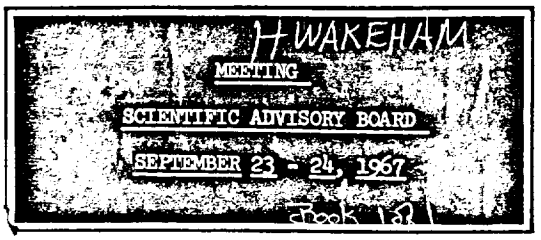


1003546610

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

TABLE OF CONTENTS

1. Agenda

2. Minutes of May meeting

3. List of administrative actions

4. List of applications to be considered

5. List of current grants by subject

6. List of current grants by activation date

7. Copies of applications

   a. Renewal applications

   b. Applications for supplements

   c. Resubmitted application (modified)

   d. New applications from present or recent grantees

   e. New applications

100354611

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

AGENDA

SCIENTIFIC ADVISORY BOARD MEETING

SEPTEMBER 23 - 24, 1967

---

1. Administrative actions taken since May 20, 1967

2. Staff Reports on matters referred by the Board

3. Consideration of applications

   a. Renewal applications

   b. Supplements requested

   c. Resubmitted (modified) application

   d. New applications from present or recent grantees

   e. New applications

4. Program planning

   Special session for staff reports on recent developments in certain projects and policy discussions of methods for effective follow-up.

5. Miscellaneous

6. Future meeting dates

10035466612

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

CONFIDENTIAL REPORT

SCIENTIFIC ADVISORY BOARD MEETING

New York, New York

May 20-21, 1967

Dr. Kenneth M. Lynch, Chairman	
Dr. C. C. Little	Scientific Director, CTR
Dr. Richard J. Bing	
Dr. McKeen Cattell	
Dr. Leon O. Jacobson	
Dr. Clayton G. Loosli	
Dr. Stanley P. Reimann	
Dr. Sheldon C. Sommers	
Dr. John W. Fertig (5/20/67)	Columbia University
Mr. W. T. Hoyt	Executive Director, CTR
Dr. Robert C. Hockett	Associate Scientific Director, CTR
Dr. J. Morrison Brady	Associate Scientific Director, CTR
Dr. John H. Kreisher	Associate Scientific Director, CTR
Dr. Vincent F. Lisanti	Scientific Associate, CTR

1. The report of actions taken at the December meeting was approved by the Board.

2. Administrative Actions

#390R2 Peter H. Knapp, Ph.D. A balance of $252.36 from the completed project was received and deposited.

#408R1 Benjamin Burrows, M.D. A balance of $1,500.00 from the completed project was received and deposited.

#466R1 E. C. Hoff, Ph.D., M.D. The grantee was authorized to use the unexpended balance of approximately $4,000.00 for the needs of the project after the termination date of May 31, 1967.

#510R1 Donald J. Massaro, M.D. This grant will be transferred to Duke University Medical Center following receipt of financial report and refund from Georgetown University.

#592 Donald J. Massaro, M.D. $7,820.00 Transferred to Duke University Medical Center.

1003546613

#528R1 Sue Buckingham, M.D. After staff negotiation, the final sum approved for this year's grant is $15,155.00 with the understanding that the money still remaining from her previous grants be used also to defray the costs of this project to a total of $31,624.00, plus $2,000.00 from carryover for contingencies.

Source: https://www.industrydocuments.ucsf.edu/docs/pfdm0000

#543R1 Ulrich Schaeppi, M.D. $17,260.00
#598 Ulrich Schaeppi, M.D. $10,175.00
The request of a transfer of both grants from The Worcester Foundation for Experimental Biology to Mason Research Institute, also of Worcester, Massachusetts, approved. The question of a contract or grant should be clarified and decided before the letter of approval is sent.

#572 T. Timothy Crocker, M.D. Permission was granted to transfer $1,000 from the personnel category to travel ($450) and supplies & services ($550).

#579 Naiter M. Chopra, Ph.D. $23,955.00 Approved. All questions have been answered and the grant will proceed.

#585 George W. Smetters, M.D. (Thomas C. Laipply, M.D.) $11,963.00 Approved. The balance of $13,879.67 remaining from previous grants is to be returned to The Council.

#591 Paul S. Larson, Ph.D. The final amount for this grant has not been agreed upon, pending Dr. Larson's discussions with publishers.

#607 Samuel Bellet, M.D. Permission was granted to transfer $800 of the project funds to travel expenses.

#609 Donald B. Louria, M.D. Proper official approval from Cornell University has been received and the grant has been authorized for activation as of July 1, 1967.

#610 Caroline Bedell Thomas, M.D. $29,865.00 A terminal one-year grant was approved in order to summarize work status and to allow transfer of such information to Mr. Kurt Enslein for evaluation. These monies are to provide for salaries to keep Dr. Thomas' group together and are not for the purpose of continuing the project indefinitely. The Board agreed that Mr. Enslein should be remunerated for this service in accord with past procedures.

#611 John R. Rowlands, Ph.D. The committee recommended placing this application on the agenda for the September meeting.

3. Supplements

#516R1S Donald M. Pace, Ph.D. $2,003.60 Approved.

#558S G. H. Friedell, M.D. $5,403.00 Approved.

#566S Kenneth M. Lynch, M.D. and Forde A. McIver, M.D. $1,200.00 Approved.

1003546614

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4. Renewal Applications

#423M2R2 Bertram Eichel, D.D.S. $29,594.00 Approved for the terminal year of a three-year program at the previous rate of $17,950.00.

#461MR1 Rose Marie Pangborn, M.S. $22,433.00 Approved.

#467R2 Thomas C. Westfall, Ph.D. $11,500.00 Approved for the terminal year of a three-year program.

#472R2 James E. P. Toman, Ph.D. $15,225.00 Approved for the first year of a three-year program.

#477MR2 Marcus N. Carroll Jr., Ph.D. $19,620.00 Approved for one year at the requested amount contingent upon assurance from the institution that his salary will be secure for the entire period of the grant.

#478R2 Stephen M. Ayres, M.D. $22,890.00 Approved for the terminal year of a three-year program.

#492R2 Walter M. Booker, Ph.D. $19,568.00 Approved for the terminal year of a three-year project at the previous rate of $17,705.00.

#513R1 Hyman Engelberg, M.D. $24,966.00 Approved for the terminal year of a two-year program. Dr. Loosli will request that Dr. Samuel Rappaport review this research program before the next meeting.

#521R1 Cesare Biancifiori, M.D. $10,100.00 Approved for the second year of a program originally submitted as a three-year plan. It was suggested that urethane be used as a control to produce carcinoma of the lung. The staff will review this program for the next meeting and a visit by a Board member is contemplated.

#526R1 Roger K. Larson, M.D. $17,424.00 Approved for the terminal year of a two-year program.

#534R1 Paul Goldhaber, D.D.S. $23,396.75 Approved for the second year of a three-year program with the understanding that any residual funds remaining from the previous year be applied against this grant total.

#546R1 Ines Mandl, Ph.D. $25,969.00 Approved for the second year of a three-year plan.

#547R1 Gustave A. Laurenzi, M.D. $12,229.00 Approved. A future conference should be developed around this program.

#548R1 S. N. Pradhan, M.D., Ph.D. $9,928.00 Approved for the second year of a three-year plan.

#554R1 Broda O. Barnes, Ph.D., M.D. $23,000.00 Denied. Dr. Loosli will contact WHO, NCI, etc., to see if an overall effort for an ongoing survey is possible.

1003546615

#555R1 Gene M. Smith, Ph.D. $14,184.00 Approved as a terminal grant.

#559R1 Sheldon C. Sommers, M.D. $14,536.00 Approved for the second year of a three-year plan.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

5. New Applications

#578M	Harry S. Bernton, M.D. $21,460.00 Denied.
#612	Jay D. Coffman, M.D. $5,192.00 Approved.
#613	R. Ernest Clark, Ph.D. $23,328.80 Denied.
#614	Maurice S. Segal, M.D. $85,650.00 Approved in the amount of $30,000.00 plus overhead. Final arrangements are to be made by staff.
#615	Charles E. Tobin, Ph.D. $7,634.85 Denied.
#616	Robert A. Kuhn, M.D. $30,072.50 Denied.
#617	Luben G. Angeloff $12,805.00 Denied.
#618	Edmond Antony Murphy, M.D., Sc.D. $8,430.00 Approved for the first year of a two-year program.
#619	H. A. I. Newman, Ph.D. $17,675.00 Denied without prejudice. The Board thought this was an excellent research proposal but not within the scope of The Council's program.
#620	A. Weinstock, Ph.D. $40,036.00 Approved as a one-year program with review by the staff at the end of nine months.
#621	Ole A. Holtermann, M.D. $12,978.00 Denied.

6. The staff was requested to look into the forms used by other granting agencies concerning research carried out on humans, and possibly incorporate a statement in the grant application forms to the effect that the investigators should abide by the Helsinki Declaration.

7. The staff will send to Dr. Ratzenhofer a copy of the Scientific Director's Report and a letter of explanation of the mission of The Council. This letter will emphasize the non-prejudicial attitude toward foreign applications for research grants.

8. The next meeting of the Scientific Advisory Board will be held in New York City on September 23-24, 1967.

100354616

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

ADMINISTRATIVE ACTIONS

[redacted]

100354661

ADMINISTRATIVE ACTIONS

Reports to Scientific Advisory Board
No Action Required

Followup

#477MR2 Marcus N. Carroll Jr., Ph.D. Word has been received from the institutional authorities that Dr. Carroll's salary has been guaranteed until June 30, 1968. His grant has, therefore, been activated effective July 1, 1967 in the amount of $19,620.00.

#510R1 Donald J. Massaro, M.D. A refund of $4,105.83 was received from Georgetown University and deposited. A new check in this amount has been issued to Duke University for continuation of Dr. Massaro's grant.

#534R1 Paul Goldhaber, D.D.S. The final sum approved for the renewal grant is $22,938.75 which, with an unexpended balance of $458.00 from the previous grant totals $23,396.75, the amount originally requested.

#614 Maurice S. Segal, M.D. The final sum authorized for the new grant is $34,500.00, representing $30,000.00 plus 15% overhead in the amount of $4,500.00.

Refunds Received

#385R2 William F. McNary Jr., Ph.D. Refund of $3,107.27 received and deposited.

#489 E. T. Angelakos, M.D., Ph.D. A refund of $247.84 received and deposited.

#506 Roger K. Larson, M.D. A refund of $486.57 received and deposited.

#585 George W. Smetters, M.D. The balance of $13,879.67 (grants #363, 450A and 450B) was returned as requested and deposited.

Reports to Scientific Advisory Board
Action Required

Time Extensions

#415R1 Duane G. Wenzel, Ph.D. A time extension was authorized for one year, without additional funds, to May 31, 1968.

#448R1 John S. Waugh, Ph.D. A time extension to June 30, 1968 and use of uncommitted balance of $11,495.42 was authorized for the purposes of the project.

#495 Walter Redisch, M.D. Permission granted to extend the grant from June 30 to September 30, 1967, and to use the uncommitted funds for the purposes of the project during that interval. A financial report was requested.

1003546618

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Budget Reallocations

#493M — Barbara K. Watson, Ph.D. Reallocation of the grant budget was authorized to cover travel expenses in the amount of $229.00.

#599 — Domingo M. Aviado, M.D. Permission was granted to reallocate the budget to provide $750.00 for travel expenses.

Carryover of Grant Funds

#409R2 — Frederik B. Bang, M.D. Permission requested to carry over balance of $5,184.67 from the 1966 grant to the current year.

#493MR1 — Barbara K. Watson, Ph.D. Permission requested to carry over balance of $5,072.88 from previous grant.

#516 — D. M. Pace, Ph.D. The grantee has requested that an estimated balance of $1,000+ be transferred from the University of Nebraska to the University of the Pacific to be used for additional personnel and equipment. A letter has been sent to the University of Nebraska requesting a financial statement and return of the excess funds.

#559R1 — Sheldon C. Sommers, M.D. Permission requested to carry over balance of $1,359.29 from previous grant.

Supplemental Approval

#455S — Charles L. Rose, M.A. A supplement in the amount of $3,300.00 was approved.

Correction

#492R2 — Walter M. Booker, Ph.D. The May minutes record the sum approved as $17,205.00 whereas the correct amount is $17,705.00.

10035466 19

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LIST OF APPLICATIONS

10035466620

Source: https://www.industrydocuments.ucsf.edu/docs/ptam0000

APPLICATIONS TO BE CONSIDERED

Grant No.	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Category	Subcommittee
Renewal Applications						
223R8	McKennis	46,205	3 years	52,598	Pharmacology ~~Chemistry~~	Cattell, Jacobson Bing
361R4	Gey	58,400 (16 months)	3 years	43,434	CANCER ~~Tissue Culture~~	Little, Lynch Reimann, Loosli, Sommers
409R3	Bang	30,609	3 years	21,835	pulmonary ~~Virus~~	Reimann, Sommers Jacobson
455R1	Rose	18,500	1 year	13,300	Epidemiology	Sommers, Cattell Little
523R1	Jones	32,428	3-5 years	10,000	pulmonary ~~Virus~~	Jacobson, Bing, Lynch, Loosli
558MR1	Friedell	31,050	1 year	36,453	CANCER ~~Clinical Pathologic~~	Reimann, Jacobson Lynch
561R1	Brinkman	12,109	2 years	15,653	Carcinogenesis	Bing, Sommers Reimann
566R1	Lynch	32,570	1 year	27,121	Carcinogenesis	Reimann, Jacobson Loosli, Sommers
567R1	Severi	15,000	2 years	16,000	Carcinogenesis	Little, Sommers Reimann, Loosli
569R1	Moser	15,980	2 years	15,137	Cardiovascular	Cattell, Bing Jacobson
573R1	Loosli	51,576	2 years	53,755	pulmonary ~~Virus~~	Jacobson, Lynch Reimann, Sommers
Supplemental Applications						
456R2	Homburger	6,000	1 year	70,000	cancer ~~Bioassay~~	Little, Jacobson Sommers, Reimann
467R2	Westfall	7,500		11,500	Pharmacology	Bing, Cattell Jacobson, Lynch
Modified Application						
613M	Clark	23,328	1 year		Epidemiology ~~Psychosomatic~~	Little, Loosli Cattell
New Applications from Present or Recent Grantees						
338-A	Homburger	55,617	5 years	59,096	pharmacology ~~Bioassay~~	Sommers, Jacobson Loosli, Reimann
626	Redisch	16,795	1 year	12,075	Cardiovascular	Bing, Cattell Jacobson
631	Domino	28,565	3 years	33,483	Pharmacology	Cattell, Jacobson Little
632	Strong	20,516	3 years	17,531	Cardiovascular	Reimann, Bing Cattell, Sommers

1003546621

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

APPLICATIONS TO BE CONSIDERED

Grant No.	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Category	Subcommittee
New Applications						
611	Rowlands	46,058	3 years	miscellaneous	~~Chemistry~~ Pharmacology	Jacobson, Cattell Bing
622	Geller	29,442	3 years		~~Psychosomatic~~	Cattell, Little Bing
623	Essman	13,570	2 years	pharmacology	~~Psychosomatic~~	Little, Cattell Jacobson
624	Noakes	30,900	3 years	miscellaneous	~~Chemistry~~	Jacobson, Loosli Reimann
625	Brown	12,548	2 years		Cardiovascular	Cattell, Bing Sommers
627	Beller	26,391	3 years		Cardiovascular	Bing, Cattell Jacobson, Lynch
628	Finley	26,105	3 years	pulmonary	~~Physiol. Respiratory System~~	Sommers, Loosli Reimann, Bing
629	Erickson	9,350	1 year		Pharmacology - Psychosomatic	Cattell, Little Jacobson
630	Parshley	20,169.50	2 years	pulmonary	~~Tissue Culture~~	Loosli, Sommers Reimann, Little
633	Boyarsky	61,950	3 years		Pharmacology	Cattell, Bing Jacobson

10035466622

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CURRENT GRANTS
BY SUBJECT

[redacted]

10035462623

Source: https://www.industrydocuments.ucsf.edu/docs/ptam0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

September 23-24, 1967

CURRENT GRANTS

(By Subject)

BIOASSAY, CARCINOGENESIS & TISSUE CULTURE

		Activated	Amount	Percent of Total Awards
Bioassay				
338	F. Homburger	2/1/67	59,096	
456	F. Homburger	1/1/67	70,000	
			129,096	9%
Carcinogenesis				
413	C. Leuchtenberger	4/1/67	55,150	
451	M. S. Protzel	2/1/67	31,353	
521	C. Biancifiori	3/1/67	10,100	
558	G. H. Friedell	11/1/66	36,453	
561	G. L. Brinkman	11/1/66	15,653	
564	J. U. Schlegel	1/1/67	23,000	
566	K. M. Lynch & F. A. McIver	10/1/66	27,121	
567	L. Severi	10/1/66	16,000	
603	S. L. Kauffman	9/1/67	18,439	
606	A. Furst	4/1/67	33,314	
			266,583	18%
Tissue Culture				
516	D. M. Pace	2/1/67	16,930	
572	T. T. Crocker	2/1/67	33,131	
			50,061	3%
			$445,740	30%

1003546624

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY & CHEMISTRY

		Activated	Amount		Percent of Total Awards
Cardiovascular					
310	R. J. Bing	7/1/67	11,261		
467	T. C. Westfall	9/1/67	11,500		
472	J. E. P. Toman	7/1/67	15,225		
477	M. N. Carroll Jr.	7/1/67	19,620		
513	H. Engelberg	7/1/67	24,966		
569	K. M. Moser	10/15/66	15,137		
607	S. Bellet	7/1/67	17,000		
612	J. D. Coffman	7/1/67	5,192		
618	E. A. Murphy	6/1/67	8,430		
			128,331		9%
Pharmacology					
206	E. F. Domino	1/1/67	33,483		
491	J. P. Long	6/1/67	8,550		
492	W. M. Booker	7/1/67	17,705		
543	U. Schaeppi	6/1/67	17,260		
548	S. N. Pradhan	8/1/67	9,928		
588	B. Bhagat	1/1/67	9,695		
598	U. Schaeppi	6/1/67	10,175		
			106,796		7%
Chemistry					
223	H. S. McKennis Jr.	7/1/66	52,598		
486	A. A. Albanese	1/1/67	13,869		
534	P. Goldhaber	7/1/67	23,396	(?)	
579	N. M. Chopra	6/1/67	23,955		
609	D. B. Louria	7/1/67	28,092		
620	A. Weinstock	6/1/67	40,036		
			181,946		12%
			$417,073		28%

10035466625

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

EPIDEMIOLOGY, PSYCHOSOMATIC & STATISTICS

		Activated	Amount	Percent of Total Awards
Epidemiology				
455	C. L. Rose	1/1/67	800	
510	D. J. Massaro	2/1/67	9,315	
576	J. H. Rogers (18 mos.)	2/1/67	15,000	
			25,115	2%
Psychosomatic				
517	A. Damon	2/1/67	13,282	
527	N. W. Heimstra (to 12/31/67)	6/1/66	9,487	
555	G. M. Smith	7/1/67	14,184	
610	C. B. Thomas	6/1/67	29,865	
			66,818	5%
			$91,933	7%
LUNG PHYSIOLOGY				
478	S. M. Ayres	7/1/67	22,890	
497	A. A. Liebow	10/1/66	9,499	
519	L. A. Soloff	2/1/67	33,600	
528	S. Buckingham	7/1/67	15,155	
546	I. Mandl	7/1/67	25,969	
547	G. A. Laurenzi	7/1/67	12,229	
565	L. S. Tsai	7/1/67	18,000	
592	D. J. Massaro	5/1/67	7,820	
599	D. M. Aviado	4/1/67	24,497	
604	R. H. Earle	7/1/67	14,970	
			$184,629	12%
PATHOLOGIC DIAGNOSTIC				
423	B. Eichel	8/1/67	17,950	
526	R. K. Larson	8/1/67	17,424	
559	S. C. Sommers	7/1/67	14,536	
585	G. W. Smetters	1/1/67	11,963	
608	W. F. McNary Jr.	6/1/67	16,906	
614	M. S. Segal	7/1/67	34,500	
			$113,279	8%

100354626

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

VIRUS, PREVIOUS INFECTIONS

		Activated	Amount	Percent of Total Awards
Virus				
409	F. B. Bang	10/1/66	21,835	
493	B. K. Watson	4/1/67	20,845	
523	O. R. Jones	10/1/66	10,000	
550	J. E. Craighead	1/1/67	16,835	
573	C. G. Loosli	10/1/66	53,755	
			$123,270	8%
ORAL CAVITY				
461	R. M. Pangborn	7/1/67	22,433	
529	J. H. Manhold (18 mos.)	4/1/66	7,430	
			$29,863	2%
MISCELLANEOUS				
24	P. S. Larson	7/1/67	23,921	
80	Fellowship Program	6/1/67	56,000	
591	P. S. Larson	?	?	
			$79,921	5%
			$1,485,708	100%

100354627

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CURRENT GRANTS
BY ACTIVATION

10035466 28

Source: https://www.industrydocuments.ucsf.edu/tobacco/

CURRENT GRANTS

(By Date of Activation)

No.	Grantee	Date	1966	1967
206	E. F. Domino	1/1		33,483
455	C. L. Rose	"		800
456	F. Homburger	"		70,000
486	A. A. Albanese	"		13,869
550	J. E. Craighead	"		16,835
564	J. U. Schlegel	"		23,000
585	G. W. Smetters	"		11,963
588	B. Bhagat	"		9,695
338	F. Homburger	2/1		59,096
451	M. S. Protzel	"		31,353
510	D. J. Massaro	"		9,315
516	D. M. Pace	"		16,930
517	A. Damon	"		13,282
519	L. A. Soloff	"		33,600
572	T. T. Crocker	"		33,131
576	J. H. Rogers (18 mos.)	"		15,000
521	C. Biancifiori	3/1		10,100
				$401,452

10035462629

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

No.	Grantee	Date	1966	1967
413	C. Leuchtenberger	4/1		55,150
493	B. K. Watson	"		20,845
529	J. H. Manhold (18 mos.)	"	7,430	
599	D. M. Aviado	"		24,497
606	A. Furst	"		33,314
592	D. J. Massaro	5/1		7,820
80	Fellowship Program	6/1		56,000
491	J. P. Long	"		8,550
527	N. W. Heimstra (to 12/31/57)	"	9,487	
543	U. Schaeppi	"		17,260
579	N. M. Chopra	"		23,955
598	U. Schaeppi	"		10,175
608	W. F. McNary Jr.	"		16,906
610	C. B. Thomas	"		29,865
618	E. A. Murphy	"		8,430
620	A. Weinstock	"		40,036
			$16,917	$352,803

100354630

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

No.	Grantee	Date	1966	1967
24	P. S. Larson	7/1		23,921
223	H. S. McKennis Jr.	"	52,598	
310	R. J. Bing	"		11,261
461	R. M. Pangborn	"		22,433
472	J. E. P. Toman	"		15,225
477	M. N. Carroll Jr.	"		19,620
478	S. M. Ayres	"		22,890
492	W. M. Booker	"		17,705
513	H. Engelberg	"		24,966
528	S. Buckingham	"		15,155
534	P. Goldhaber	"	$18,869.75	[redacted]
546	I. Mandl	"		25,969
547	G. A. Laurenzi	"		12,229
555	G. M. Smith	"		14,184
559	S. C. Sommers	"		14,536
565	L. S. Tsai	"		18,000
604	R. H. Earle	"		14,970
607	S. Bellet	"		17,000
609	D. B. Louria	"		28,092
612	J. D. Coffman	"		5,192
614	M. S. Segal	"		34,500
423	B. Eichel	8/1		17,950
526	R. K. Larson	"		17,424
548	S. N. Pradhan	"		9,928

10035466631

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

No.	Grantee	Date	1966	1967
467	T. C. Westfall	9/1		11,500
591	P. S. Larson	?		?
603	S. L. Kauffman	"		18,439
			$52,598	$456,485

1003546632

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

No.	Grantee	Date	1966	1967
409	F. B. Bang	10/1	21,835	
497	A. A. Liebow	"	9,499	
523	O. R. Jones	"	10,000	
566	K. M. Lynch & F. A. McIver	"	27,121	
567	L. Severi	"	16,000	
573	C. G. Loosli	"	53,755	
569	K. M. Moser	10/15	15,137	
558	G. H. Friedell	11/1	36,453	
561	G. L. Brinkman	"	15,653	
			$205,453	

10035466633

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

RENEWALS

10035466634

Source: https://www.industrydocuments.ucsf.edu/docs/pbmh0000

#223R8-McKENNIS—

1003546635

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

July 25, 1967

TO: The committee comprising Dr. Cattell, Chm., Dr. Jacobson and Dr. Bing.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Herbert McKennis, Jr., Ph.D. - No. 223-R8.

We enclose herewith a renewal application from Dr. Herbert McKennis, Jr., of the Medical College of Virginia, Richmond, Virginia. His covering letter which describes the change in emphasis to biological and enzymatic studies, is included. In the past the grant has been renewed on an annual basis, but it should be noted that the present proposal anticipates a three-year study, with support at approximately the same rate as we provided in recent years.

This has been a very worthwhile and productive program, which the Council has supported for the past eleven years.

When the grant was renewed last year it was emphasized that we would not be able to maintain support for studies on nicotine metabolites indefinitely (a point also raised the year before), and it was indicated that we would need to scale down appropriations for work in this area.

R. C. H.

1003546636

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

MEDICAL COLLEGE OF VIRGINIA

DEPARTMENT OF PHARMACOLOGY

TELEPHONE 644-9851
AREA CODE 703

RICHMOND, VIRGINIA 23219

Rec'd 7/24/67

July 12, 1967

Dr. Robert C. Hockett
Associate Scientific Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue
New York, N. Y. 10017

Dear Bob:

I am enclosing an application that describes in a brief way research thoughts in the general area of nicotine metabolism. We are very tardy in submitting this application. The reason, in part, has been our desire to run through some preliminary or range-finding experiments and to establish a feeling that we are on the right course and had developed sufficiently workable techniques so that contemplated work could proceed without delay. We now feel that this is the case and hope that the Council will see fit to consider our application, which in many ways is a continuation, with more of an emphasis on biological and enzymatic studies -- in contrast to much of the earlier work in which we concentrated on the problems of structure and syntheses of many of the nicotine metabolites.

To bring you up-to-date in an informal way on some of the more recent happenings that have not yet been reduced to manuscript or report form a few comments will be made.

Preliminary experiments were conducted on the effect of age on the ability of rat-microsomal preparations to metabolize nicotine. Using our younger rats (150-200g) as a standard it appeared quite consistently that equivalent weights of the liver fractions from the older animals (400-500g) showed a lesser ability to metabolize nicotine. Studies to relate this apparent deficit to the composition of the liver fraction and comparative studies on the ability of animals of varying ages to metabolize labelled nicotine are contemplated. Isotopic material is already on hand for these studies.

At earlier dates we talked about the progress made on the electron-capture studies with pentafluropropionylmetanicotine that had been derived from nicotine and pentafluropropionic acid anhydride as a basis for the quantitative determination of nicotine (with support from the AMA Education and Research Foundation). The major drawbacks to the analytical method, in our goal to reach 1×10^{-12} grams included a broad peak which could not be sharpened by

1003546637

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

increasing column temperature because of the background or trash that appeared in biological material. This problem seems to have been overcome in part by modification of the injection part of the apparatus (a standard procedure). Additionally, the basic chemistry of the reaction has been extended to nornicotine. The analytical sample of pentafluropropionylmetanicotine (by analysis) shows a retention time different from that of the corresponding nicotine derivative. In consequence we feel more and more secure about the method.

In the midst of other things we are setting up to run kinetic studies on the conversion of γ-3-pyridyl-γ-methylaminobutyric acid to cotinine. At least at physiological pH ranges this should be relatively simple to accomplish polarimetrically in view of the marked differences in the specific rotations of the two compounds. The data should help persuade people of the care needed in isolating cotinine.

We shall be grateful as always for any consideration given to our application. A copy with the signature of the college business officer will follow. Please do not hesitate to write or phone in the event of any questions.

We are grateful for the generous and helpful support of the past and close with our best personal wishes.

Sincerely,

Herbert McKennis, Jr.

HMcK/bb

10035466638

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY, CHEMISTRY

No. 223-R8
Activated: 7/1/59
(Compare #1 - #128)
Larson - 7/1/56 - 7/1/58.

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Jacobson
Dr. Bing

Application For Research Grant

Date:

1. Name of Investigator(s): (include Title and Degrees) Herbert McKennis, Jr., Ph.D., Professor of Pharmacology

2. Institution & Address: Medical College of Virginia, Richmond, Virginia 23219

3. Short Title of Project: Factors Controlling the Biological Disposition of Pyridine Compounds of Tobacco Smoke

4. Proposed Starting Date: July 1, 1967

5. Anticipated Duration of this Specific Study: 1967-1970 (3 years)

6. Brief Descripton of Objectives or Specific Aims:

From a series of experiments with support of the Council for Tobacco Research-U.S.A., it has been possible to describe the broad outlines of many of the routes involved in the mammalian metabolism of nicotine, nornicotine, and 3-acetylpyridine -- three of the many pyridine compounds that occur in tobacco and tobacco smoke. [The general nature of the previous studies are given in the publications which are enumerated in an attached list.] Through isolation or total synthesis it has been possible to supply many of the mammalian metabolites to biological investigators and to assist them in a variety of work on the pharmacological properties of many nicotine metabolites.

It is now desirable for us and interesting to us to expand our knowledge of the factors which determine the disposition of pyridine compounds arising from tobacco smoke and explore biochemical interactions between smoke components. There is already in the published literature some data that suggests that desensitization or tachyphylaxis to nicotine arises in part through a stimulation of the enzymes that are concerned with the metabolism of nicotine. It has been suggested that this stimulation of metabolism is produced by nicotine and that other substances have a similar capacity to enhance the metabolism of nicotine. Direct studies on nicotine metabolism are however very limited in their scope.

Through the current availability of synthetic routes to nicotine metabolites, which we have already described, and through synthetic routes which make possible the labelling of nicotine and related in almost any desired position, we believe that it is now possible to determine the nature of stimulation or inhibition of metabolism at almost all of the key points that are involved in mammalian systems. Similarly, it should be possible to determine individual differences in the capacity to metabolize nicotine and related pyridine compounds. (continued on sheet 1a attached)

7. Give a Brief Statement of your Working Hypothesis:

Current data on the effect of various smoke components on the metabolism and transfer of nicotine and other alkaloids are limited or apparently contradictory. A study of these factors should be helpful to an understanding of the biological effects of tobacco smoking.

1003546639

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

6. Brief Description of Objectives or Specific Aims: (continued)

In the case of some of the simpler metabolites of nicotine, such as methylamine, it is relatively easy to obtain a broad picture of the nature of compounds which interfere with metabolic events. Work with ^{14}C-methylamine in our own laboratory (The Pharmacologist, Fall 1967, in press) and others illustrates that interference with oxidation of methylamine may be produced by some types of monoamine-oxidase inhibitors. The data suggest, however, that the inhibition may be referrable to a so-called methylamine oxidase which has not been fully characterized. Liver preparations obtained from rats after administration of monoamine-oxidase inhibitors have thus far been shown to be as fully capable as control livers in tests which involve a spectrophotometric measurement of the disappearance of nicotine. From another series of experiments, the data now suggests that the oxidation of nicotine can be impeded by the presence of large quantities of cotinine. It would be desirable to study possible effects of other pyridine compounds of smoke on the metabolism of nicotine and related substances. The need for a biologically oriented study appears to be re-emphasized by apparently conflicting chemical data on the oxidation of nicotine. For instance, the pyrolidine-N-oxide of nicotine has been considered (by J. C. Craig) to be the logical key intermediate in the biological formation of cotinine, while Linnell has reported that the same oxide in vitro inhibits the oxidation of nicotine to cotinine.

1003546640

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

With the aid of isotopically labelled nicotine and its metabolites it is feasible to study by a number of different methods factors influencing the disposition of nicotine. It is anticipated that during the current studies both in vivo and in vitro methods will be employed.

Initial experiments to determine the suitability of the rat as an experimental animal for initial studies have already been conducted. Liver microsomal preparations from both young and old rats of the Wistar strain have been examined for their capacity to metabolize nicotine in vitro. The general picture currently presented is that equivalent fractions by weight of liver that are obtained from older animals (250-550g body weight) do not metabolize nicotine as effectively as do the respective fractions from younger animals (150-175 g body weight). It is not currently known whether or not the observed deficiency in the older animals can be attributed to a lesser amount of metabolizing cellular fractions per unit of liver weight, or an overall lessening of metabolic ability that exists throughout the animal. It would be desirable to settle this point and to determine whether or not the elimination of unchanged nicotine by the older animals suffers any impairment. The additional matter of elimination can be settled without modification of the experimental design of the metabolic experiments. Questions of this type can be answered by comparing the ability of groups of young and old animals to metabolize

(continued on sheet 2a attached)

9. Physical Facilties Available (Where Other than Administering Organization Indicate Geographical Location)

Laboratories and animal facilities are located on the fourth and fifth floors of McGuire Hall at the Medical College of Virginia. Gas chromatography and isotope counting facilities are available in areas convenient to the working laboratories but separate from these. The equipment list includes a preparative ultracentrifuge, polarimeter, Warburg apparatus, hydrogenation apparatus, and the general equipment of chemical and biological laboratories.

10. Additional Requirements:

Additional requirements, if any, would depend upon the outcome of the research.

11. Biographical sketches of all principal and professional personnel (append)

12. List of publications: (Five most recent as pertinent) (append)

100354664

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (continued)

^{14}C-labelled nicotine and to eliminate unchanged alkaloid. For all of this isotopic material is now available as a result of our synthetic work and previous nutriculture: nicotine-^{14}C (random), nicotine-^{14}C-methyl, and nicotine-2-^{14}C. In addition, six of the mammalian metabolites of (-)-nicotine are available in both isotopic and non-isotopic form so that quantitative data can be obtained.

1003546642

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Edward R. Bowman, Ph.D.	100%	
Research Fellow (Ph.D. organic)	100%	
Technical		
Laboratory Assistant, B.S.	100%	
Laboratory Aide	100%	
Sub-Total:		
B. Consumable Supplies (list by categories)		
Chemical Reagents		2,200
Isotopes		1,500
Glass ware		800
Animals and feed		750
Sub-Total:		5,250
C. Other Expenses (itemize)		
Reprints and page charges		250
Travel (Presentation of reports at meetings and conferences)		500
Sub-Total		750
D. Permanent Equipment (itemize)		
Vacuum pumps (2)		550
		550
E. Overhead (15% of A+B+C)		5,955
Total:		46,205

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R R	5,500	1,000	1,000	6,675	52,175
Year 3		6,000	1,000	1,000	7,050	55,050

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature] Director of Project

644-9851 xt. 8153 Telephone

Signature [signature] Business Officer of the Institution

L. DANIEL COOKS Telephone
VICE PRESIDENT – BUSINESS AND FINANCE

10035466643

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Quantitative Methods for the Determination of the Distribution of Nicotine and Its Congeners in Biological Systems Institution supplies salary of principal investigator and all secretarial help currently obtained through other funds	American Medical Association Education and Research Foundation	$39,785.00	Expires January, 19[illegible]

Pending

None other than this application for the Council for Tobacco Research - U.S.A.

1003546644

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Biographical Information

on

HERBERT McKENNIS, JR.

R R R Attended elementary schools in Scarsdale, N.Y. and Loomis School, Windsor, Conn., Harvard S.B., R and Cornell Ph.D., R

Chemist at Nuodex Products Co., 1938-1939, and Ciba Pharmaceutical Products Co., 1940-1942.

Appointed Assistant in Biochemistry, Cornell University Medical College, 1942; Assistant Professor of Chemistry, Medical College of Virginia, 1945; Instructor in Physiological Chemistry, the Johns Hopkins University, 1946; Associate Professor of Biochemistry, Medical College of Virginia, 1948; Head, Basic Sciences Research Department, Naval C. E. Laboratory, 1949; Associate Professor, 1953, Professor of Pharmacology, Medical College of Virginia, 1955.

Biochemist; specialist in intermediary metabolism, antibiotics, alkaloids, polymeric compounds. U.S. and foreign patents: soil stabilization and antispasmodic.

Has done a wide variety of scientific advisory and technical consulting work for industrial firms, government agencies, scientific organizations, and universities.

Visiting Professor, University of Chile, 1960; Honorary member, Faculty of Medicine, University of Chile, Sociedad de Biologia de Santiago. Sigma Xi. Phi Lambda Upsilon.

American Chemical Society, American Society of Biological Chemists, The Society for Experimental Biology and Medicine, International Oceanographic Foundation, The New York Academy of Sciences, American Society for Pharmacology and Experimental Therapeutics, American Association for the Advancement of Science, Society of American Military Engineers, American Institute of Chemists, Virginia Academy of Science, Society of Toxicology.

1003546645

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Edward R. Bowman
Medical College of Virginia
Richmond, Virginia

Born: West Virginia 1927
Citizen U.S.A.

Education:

Concord College B.S. 1952 (Biology - Chemistry).

West Virginia University 1953 (Physiology).

Duke University 1955-56 (Graduate Student in Physiology).

Medical College of Virginia Ph.D. 1963 (Pharmacology).

Experience:

1961 - present	Research Associate Department of Pharmacology Medical College of Virginia Richmond, Virginia
1958 - 1961	Graduate Student, Major - Pharmacology Minor - Physiology & Biochemistry Medical College of Virginia Richmond, Virginia
1956 - 1958	Research Assistant Department of Pharmacology Medical College of Virginia Richmond, Virginia
1955 - 1956	Graduate Student, Major - Physiology Minor - Anatomy Duke University Durham, North Carolina
1954 - 1955	Bacteriologist State Department of Health Richmond, Virginia
1952 - 1953	Graduate Student, Physiology West Virginia University Morgantown, West Virginia
1952	Student, Biology & Chemistry Concord College Athens, West Virginia
1950 - 1951	U.S. Army
1947 - 1950	Student, Biology & Chemistry Concord College Athens, West Virginia
1944 - 1946	U.S. Army

100354646

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REDACTED

REDACTED

REDACTED

REDACTED

100354664?

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS

OF PROJECT WORK SUPPORTED BY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

1. Synthesis and properties of pyridylalanines.
   Herbert McKennis, Jr., and Edward R. Bowman.
   Virginia Journal of Science, 8, 314 (1957).

2. Metabolism of γ-(3-pyridyl)-γ-oxobutyric acid.
   Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
   Federation Proceedings, 17, 325 (1958).

3. γ-(3-Pyridyl)-γ-methylaminobutyric acid as a urinary metabolite of nicotine.
   Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
   Journal of the American Chemical Society, 79, 6342 (1957).

4. Metabolites of nicotine and a synthesis of nornicotine.
   Herbert McKennis, Jr., Lennox B. Turnbull, Harvey N. Wingfield, Jr., and Lovell J. Dewey.
   Journal of the American Chemical Society, 80, 1634 (1958).

5. The role of cotinine in nicotine metabolism
   Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
   Abstracts of Communications, IV. International Congress of Biochemistry, Vienna, Sept. 1-6, 1958.

6. A constant rate infusion apparatus.
   Quentin S. McKennis, Edward R. Bowman, and Herbert McKennis, Jr.
   Toxicology and Applied Pharmacology, 1, 61 (1959).

7. Metabolism of nicotine to (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid.
   Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
   Journal of the American Chemical Society, 80, 6597 (1958).

8. Metabolism of nicotine in the human and excretion of pyridine compounds by smokers.
   Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
   The Journal of Pharmacology and Experimental Therapeutics, 127, 92 (1959).

9. Demethylation of cotinine in vivo.
   Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Einosuke Wada.
   Journal of the American Chemical Society, 81, 3951 (1959).

10. Oxidation of cotinine in vivo.
    Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
    Federation Proceedings, 18, 371 (1959).

100354664

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Depressor activity from nicotine metabolites.
Edward R. Bowman, H. B. Kennedy, Jr., Einosuke Wada, and Herbert McKennis, Jr.
Abstracts of Communications, XXI. International Congress of Physiological Sciences, 41, Buenos Aires, August 9-15, 1959.

12. The isolation and structure of a ketoamide formed in the metabolism of nicotine.
Herbert McKennis, Jr., Edward R. Bowman, and Lennox B. Turnbull.
Journal of the American Chemical Society, 82, 3974 (1960).

13. Methylation in the metabolism of (-)-nicotine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 19, 268 (1960).

14. The excretion and metabolism of nicotine.
Herbert McKennis, Jr.
Annals of the New York Academy of Sciences, 90, 36 (1960).

15. Norcotinine (desmethylcotinine) as a urinary metabolite of nornicotine.
Einosuke Wada, Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
Journal of Medicinal and Pharmaceutical Chemistry, 4, 21 (1961).

16. Depressor effects arising from (-)-cotinine.
Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
The Pharmacologist, 2, 72 (1960).

17. Oxidation of nicotine-C-14 and nicotine-methyl-C-14 in vivo.
Lennox B. Turnbull, Einosuke Wada, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 20, 172 (1961).

18. Metabolism of nicotine in the human.
Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
Virginia Journal of Science, 9, 438 (1948).

19. Degradation of (-)-cotinine in the human.
Herbert McKennis, Jr., and Edward R. Bowman.
Abstracts of Communications, V. International Congress of Biochemistry, Moscow, August 10-16, 1961, p. 393.

20. The mammalian degradation of (-)-nicotine to 3-pyridylacetic acid and other compounds.
Herbert McKennis, Jr., Edward R. Bowman, and Lennox B. Turnbull.
Proceedings of the Society for Experimental Biology and Medicine, 107, 145 (1961).

21. Metabolism of (-)-cotinine in the rat.
Sorell L. Schwartz, Edward R. Bowman, and Herbert McKennis, Jr.
Virginia Journal of Science, 12, 196 (1961).

10035466649

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

22. Demethylation in the metabolism of (-)-nicotine.
Herbert McKennis, Jr., Lennox B. Turnbull, Sorell L. Schwartz, Einosuke Tamaki, and Edward R. Bowman.
The Journal of Biological Chemistry, 237, 541 (1962).

23. Metabolism of (-)-cotinine to a keto acid.
Sorell L. Schwartz, and Herbert McKennis, Jr.
Federation Proceedings, 21, 183 (1962).

24. Studies on the respiratory and cardiovascular effects of (-)-cotinine.
Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
The Journal of Pharmacology and Experimental Therapeutics, 137, 313 (1962).

25. Studies on the metabolism of (-)-cotinine in the human.
Edward R. Bowman, and Herbert McKennis, Jr.
The Journal of Pharmacology and Experimental Therapeutics, 135, 306 (1962).

26. Routes in the mammalian metabolism of (-)-nicotine to 3-pyridylacetic acid.
Sorell L. Schwartz, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 16th Tobacco Chemists' Research Conference, Richmond, Virginia, Sept. 26-28, 1962, p. 7.

27. Studies on the metabolic fate of 3-acetylpyridine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 16th Tobacco Chemists' Research Conference, Richmond, Virginia, Sept. 26-28, 1962, p. 6.

28. The corrected structure of a ketoamide arising from the metabolism of (-)-nicotine.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Sorell L. Schwartz.
Journal of the American Chemical Society, 84, 4598 (1962).

29. N-Methylation of nicotine and cotinine in vivo.
Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
The Journal of Biological Chemistry, 238, 719 (1963).

30. Studies on the degradation of the pyrrolidine ring of (-)-nicotine in vivo. Formation of γ-(3-pyridyl)-γ-oxobutyric acid.
Sorell L. Schwartz, and Herbert McKennis, Jr.
The Journal of Biological Chemistry, 238, 1807 (1963).

31. The synthesis of hydroxycotinine and studies on its structure.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Einosuke Tamaki.
Journal of Organic Chemistry, 28, 383 (1963).

32. (-)-Cotinine.
Edward R. Bowman, and Herbert McKennis, Jr.
Biochemical Preparations, 10, 36 (1963).

10035466650

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

33. Conjugate formation in the metabolism of 3-acetylpyridine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 17th Tobacco Chemists' Research Conference, Montreal, Quebec, Canada, Sept. 22-25, 1963, p. 11.

34. A comparative study of the metabolic release of methyl groups from a series of N-methylpyridinium compounds.
Herbert McKennis, Jr., Edward R. Bowman, Antonio Horvath, and John P. Bederka, Jr.
Nature, 202, 699 (1964).

35. Mammalian degradation of (-)-demethylcotinine.
Sorell L. Schwartz, and Herbert McKennis, Jr.
Nature, 202, 594 (1964).

36. Disposition and fate of (-)-cotinine-H^3 in the mouse.
Edward R. Bowman, Eskil Hansson, Lennox B. Turnbull, Herbert McKennis, Jr., and Carl G. Schmiterlöw.
The Journal of Pharmacology and Experimental Therapeutics, 143, 301 (1964).

37. Additional routes in the metabolism of 3-acetylpyridine.
Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 1215 (1964).

38. Effect of cotinine and other nicotine metabolites in vitro on duodenum and ileum segments.
K. S. Kim, Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 23, 330 (1964).

39. The metabolic formation of γ-(3-pyridyl)-γ-hydroxybutyric acid and its possible intermediary role in the mammalian metabolism of nicotine.
Herbert McKennis, Jr., Sorell L. Schwartz, Lennox B. Turnbull, Einosuke Tamaki, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 3981 (1964).

40. Alternate routes in the metabolic degradation of the pyrrolidine ring of nicotine.
Herbert McKennis, Jr., Sorell L. Schwartz, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 3990 (1964).

41. Metabolism of 3-acetylpyridine to an analog of mandelic acid.
Lennox B. Turnbull, C. N. Lukhard, and Herbert McKennis, Jr.
Toxicology and Applied Pharmacology, 6, 362 (1964).

42. Disposition and fate of nicotine in animals.
Herbert McKennis, Jr.
Tobacco Alkaloids and Related Compounds, U. S. Von Euler, editor, Pergamon Press, Oxford, 1965, p. 53.

1003546651

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

43. Urinary excretion of conjugate forms of 1-(3-pyridyl)ethanol after administration of 3-acetylpyridine.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and C. Norman Lukhard.
The Journal of Biological Chemistry, 241, 1878 (1966).

44. The structure of dibromoticonine, a bromination product of nicotine.
Herbert McKennis, Jr., Edward R. Bowman, L. D. Quin, and R. C. Denney.
Abstracts of Papers, 152nd Meeting, American Chemical Society, New York, N. Y., Sept. 12-16, 1966.

45. Studies on the synthesis and metabolism of 4-(3-pyridyl)-4-methylaminobutyric acid-4-^{14}C.
Paolo L. Morselli, Edward R. Bowman, Helen H. Ong, and Herbert McKennis, Jr.
Virginia Journal of Science, 17, 345 (1966).

46. The fate and distribution of 1-(3-pyridyl)ethanol methiodide in relation to the toxicity of 1-(3-pyridyl)ethanol and 3-acetylpyridine.
John P. Bederka, Jr., Eskil Hansson, Edward R. Bowman, and Herbert McKennis, Jr.
Biochemical Pharmacology, 16, 1 (1967).

47. Studies on the separation of acidic metabolites of nicotine by gas chromatography.
Herbert McKennis, Jr., Edward R. Bowman, and Mohammad Saeed Dar.
Virginia Journal of Science, 18, 13 (1967).

48. Pharmacological action and intermediary role of 5-(3-pyridyl)tetrahydrofuranone-2.
Edward R. Bowman, Pavol Hrdina and Herbert McKennis, Jr.
Federation Proceedings, 26, 616 (1967).

49. Metabolism of (±)-Cotinine-2-^{14}C in the rat.
Paolo L. Morselli, Helen H. Ong, Edward R. Bowman, and Herbert McKennis, Jr.
Journal of Medicinal Chemistry, in press.

50. Methylamine metabolism in normal and mao-inhibitor-treated rats.
Paolo L. Morselli, Edward R. Bowman, and Herbert McKennis, Jr.
The Pharmacologist, in press.

10035466 52

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

51. On the congeners of whiskey.
Herbert McKennis, Jr., and Harvey B. Haag.
Journal of the American Geriatrics Society, 7, 848 (1959).

52. Depressor effects arising from (-)-cotinine.
Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
The Pharmacologist, 2, 72 (1960).

53. The excretion and metabolism of nicotine.
Herbert McKennis, Jr.
Annals of the New York Academy of Sciences, 90, 36 (1960).

54. The isolation and structure of a ketoamide formed in the metabolism of nicotine.
Herbert McKennis, Jr., Edward R. Bowman, and Lennox B. Turnbull.
Journal of the American Chemical Society, 82, 3974 (1960).

55. Methylation in the metabolism of (-)-nicotine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 19, 268 (1960).

56. L-γ-Glutamylhydrazine and the metabolism of hydrazine.
Herbert McKennis, Jr., Allan S. Yard, Elizabeth J. Adair, and J. H. Weatherby.
The Journal of Pharmacology and Experimental Therapeutics, 131, 152 (1961).

57. Demethylation in the metabolism of (-)-nicotine *in vivo*.
Herbert McKennis, Jr., Einosuke Wada, Edward R. Bowman, and Lennox B. Turnbull.
Nature, 190, 910 (1961).

58. Norcotinine (Desmethylcotinine) as a urinary metabolite of nornicotine.
Einosuke Wada, Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
Journal of Medicinal and Pharmaceutical Chemistry, 4, 21 (1961).

59. The isolation of 3-pyridylacetic acid, a urinary metabolite of (-)-cotinine.
Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
Abstracts of Papers, 14th Tobacco Chemists' Research Conference, Oct. 13-14, 1960, Winston-Salem, North Carolina.

60. Oxidation of nicotine-C^{14} and nicotine-methyl-C^{14} *in vivo*.
Lennox B. Turnbull, Einosuke Wada, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 20, 172 (1961).

61. Inhibition by thyroxine of γ-aminobutyrate-α-ketoglutarate transaminase.
Antonio Horvath, Fernando Orrego, and Herbert McKennis, Jr.
Federation Proceedings, 20, 5 (1961).

1003546653

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

62. Factors controlling the metabolism of γ-aminobutyric acid.
Antonio Horvath, Fernando Orrego, and Herbert McKennis, Jr.
The Journal of Pharmacology and Experimental Therapeutics, 134, 222 (1961).

63. Mammalian degradation of (-)-nicotine to 3-pyridylacetic acid and other compounds.
Herbert McKennis, Jr., Edward R. Bowman, and Lennox B. Turnbull.
Proceedings of the Society for Experimental Biology and Medicine, 107, 145 (1961).

64. Selective toxicity. (Book review).
Herbert McKennis, Jr.
Revista Medica de Chile, 88, 864 (1960).

65. Los oxoesteroides, el uso de hidrazidos fenólicos para su detección, caracterización y medición. (Book review).
Herbert McKennis, Jr.
Revista Medica de Chile, 88, 626 (1960).

66. Metabolism of nicotine in the human.
Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr.
Virginia Journal of Science, 9, 438 (1958).

67. Metabolism of (-)-cotinine in the rat.
Sorell L. Schwartz, Edward R. Bowman, and Herbert McKennis, Jr.
Virginia Journal of Science, 12, 196 (1961).

68. Degradation of (-)-cotinine in the human.
Herbert McKennis, Jr., and Edward R. Bowman.
Abstracts of Communications, V. International Congress of Biochemistry, Moscow, August 10-16, 1961, p. 393.

69. Aspects of the metabolism of isoniazid and acetylisoniazid in the human and the dog.
Allan S. Yard, and Herbert McKennis, Jr.
Journal of Medicinal and Pharmaceutical Chemistry, 5, 196 (1962).

70. Demethylation in the metabolism of (-)-nicotine.
Herbert McKennis, Jr., Lennox B. Turnbull, Sorell L. Schwartz, Einosuke Tamaki, and Edward R. Bowman.
The Journal of Biological Chemistry, 237, 541 (1962).

71. Inhibition of the catabolism of aspartic-4-C^{14} acid by thyroxine in vivo.
Antonio Horvath, and Herbert McKennis, Jr.
Enzymologia, 24, 91 (1962).

72. Studies on the metabolism of (-)-cotinine in the human.
Edward R. Bowman, and Herbert McKennis, Jr.
The Journal of Pharmacology and Experimental Therapeutics, 135, 306 (1962).

100354654

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

73. Metabolism of (-)-cotinine to a keto acid.
Sorell L. Schwartz, and Herbert McKennis, Jr.
Federation Proceedings, 21, 183 (1962).

74. The excretion and metabolism of triethylene glycol.
Herbert McKennis, Jr., Robert A. Turner, Lennox B. Trunbull, and Edward R. Bowman.
W. W. Muelder, M. P. Neidhardt, and Carl L. Hake.
Richard Henderson, Herbert G. Nadaeu, and Samuel Spencer.
Toxicology and Applied Pharmacology, 4, 411 (1962).

75. Studies on the respiratory and cardiovascular effects of (-)-cotinine.
Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
The Journal of Pharmacology and Experimental Therapeutics, 137, 313 (1962).

76. Routes in the mammalian metabolism of (-)-nicotine to 3-pyridylacetic acid.
Sorell L. Schwartz, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 16th Tobacco Chemists' Research Conference, Sept. 26-28, 1962, Richmond, Virginia, p. 7.

77. Studies on the metabolic fate of 3-acetylpyridine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 16th Tobacco Chemists' Research Conference, Sept. 26-28, 1962, Richmond, Virginia, p. 6.

78. Acetylhydrazine as an intermediate in the metabolism of aroylhydrazines.
Lennox B. Turnbull, Allan S. Yard, and Herbert McKennis, Jr.
Journal of Medicinal and Pharmaceutical Chemistry, 5, 1327 (1962).

79. N-Methylation of nicotine and cotinine in vivo.
Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
The Journal of Biological Chemistry, 238, 719 (1963).

80. The synthesis of hydroxycotinine and studies on its structure.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Einosuke Tamaki.
Journal of Organic Chemistry, 28, 383 (1963).

81. The corrected structure of a ketoamide arising from the metabolism of (-)-nicotine.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Sorell L. Schwartz.
Journal of the American Chemical Society, 84, 4598 (1962).

82. (-)-Cotinine.
Edward R. Bowman, and Herbert McKennis, Jr.
Biochemical Preparations, 10, 36 (1963).

1003546655

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

83. Studies on the degradation of the pyrrolidine ring of (-)-nicotine in vivo. Formation of γ-(3-pyridyl)-γ-oxobutyric acid.
Sorell L. Schwartz, and Herbert McKennis, Jr.
The Journal of Biological Chemistry, 238, 1807 (1963).

84. Conjugate formation in the metabolism of 3-acetylpyridine.
Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr.
Abstracts of Papers, 17th Tobacco Chemists' Research Conference, Sept. 22-25, 1963, Montreal, Quebec, p. 11.

85. Metabolic release of methyl groups from a series of N-methylpyridinium compounds.
Herbert McKennis, Jr., Edward R. Bowman, Antonio Horvath, and John P. Bederka, Jr.
Nature, 202, 699 (1964).

86. Mammalian degradation of (-)-demethylcotinine.
Sorell L. Schwartz, and Herbert McKennis, Jr.
Nature, 202, 594 (1964).

87. Disposition and fate of (-)-cotinine-H^3 in the mouse.
Edward R. Bowman, Eskil Hansson, Lennox B. Turnbull, Herbert McKennis, Jr., and Carl G. Schmiterlöw.
The Journal of Pharmacology and Experimental Therapeutics, 143, 301 (1964).

88. Additional routes in the metabolism of 3-acetylpyridine.
Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 1215 (1964).

89. The metabolic formation of γ-(3-pyridyl)-γ-hydroxybutyric acid and its possible intermediary role in the mammalian metabolism of nicotine.
Herbert McKennis, Jr., Sorell L. Schwartz, Lennox B. Turnbull, Einosuke Tamaki, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 3981 (1964).

90. Alternate routes in the metabolic degradation of the pyrrolidine ring of nicotine.
Herbert McKennis, Jr., Sorell L. Schwartz, and Edward R. Bowman.
The Journal of Biological Chemistry, 239, 3990 (1964).

91. Effect of cotinine and other nicotine metabolites in vitro on duodenum and ileum segments.
K. S. Kim, Joseph F. Borzelleca, Edward R. Bowman, and Herbert McKennis, Jr.
Federation Proceedings, 23, 330 (1964).

92. Metabolism of 3-acetylpyridine to an analog of mandelic acid.
Lennox B. Turnbull, C. N. Lukhard, and Herbert McKennis, Jr.
Toxicology and Applied Pharmacology, 6, 362 (1964).

1003546656

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

93. Disposition and fate of nicotine in animals.
Herbert McKennis, Jr.
Tobacco Alkaloids and Related Compounds, U. S. Von Euler, editor, Pergamon Press, Oxford, 1965, p. 53.

94. Viewpoints of the study of drug toxicity. (Book review).
Herbert McKennis, Jr.
American Journal of Pharmaceutical Education, 28, 469 (1964).

95. Urinary excretion of conjugate forms of 1-(3-pyridyl)ethanol after administration of 3-acetylpyridine.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and C. Norman Lukhard.
The Journal of Biological Chemistry, 241, 1878 (1966).

96. The structure of dibromoticonine, a bromination product of nicotine.
Herbert McKennis, Jr., Edward R. Bowman, L. D. Quin, and R. C. Denney.

10035466S7

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Papers Published

During the Period of This Report

1. Urinary excretion of conjugate forms of 1-(3-pyridyl)ethanol after administration of 3-acetylpyridine.
Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and C. Norman Lukhard.
The Journal of Biological Chemistry, 241, 1878 (1966).

2. The structure of dibromoticonine, a bromination product of nicotine.
Herbert McKennis, Jr., Edward R. Bowman, L. D. Quin, and R. C. Denney.
Abstracts of Papers, 152nd Meeting, American Chemical Society, New York, N. Y., Sept. 12-16, 1966.

3. Studies on the synthesis and metabolism of 4-(3-pyridyl)-4-methylaminobutyric acid-4-^{14}C.
Paolo L. Morselli, Edward R. Bowman, Helen H. Ong, and Herbert McKennis, Jr.
Virginia Journal of Science, 17, 345 (1966).

4. The fate and distribution of 1-(3-pyridyl)ethanol methiodide in relation to the toxicity of 1-(3-pyridyl)ethanol and 3-acetylpyridine.
John P. Bederka, Jr., Eskil Hansson, Edward R. Bowman, and Herbert McKennis, Jr.
Biochemical Pharmacology, 16, 1 (1967).

5. Studies on the separation of acidic metabolites of nicotine by gas chromatography.
Herbert McKennis, Jr., Edward R. Bowman, and Mohammad Saeed Dar.
Virginia Journal of Science, 18, 13 (1967).

6. Pharmacological action and intermediary role of 5-(3-pyridyl)tetrahydrofuranone-2.
Edward R. Bowman, Pavol Hrdina and Herbert McKennis, Jr.
Federation Proceedings, 26, 616 (1967).

7. Metabolism of (±)-cotinine-2-^{14}C in the rat.
Paolo L. Morselli, Helen H. Ong, Edward R. Bowman, and Herbert McKennis, Jr.
Journal of Medicinal Chemistry, in press.

8. Methylamine metabolism in normal and mao-inhibitor-treated rats.
Paolo L. Morselli, Edward R. Bowman, and Herbert McKennis, Jr.
The Pharmacologist, in press.

10035466658

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Talks Given

During the Period of this Report

1. Studies on the synthesis and metabolism of 4-(3-pyridyl)-4-methylaminobutyric acid-4-^{14}C. Paolo L. Morselli, Edward R. Bowman, Helen H. Ong, and Herbert McKennis, Jr. Annual Meeting of the Virginia Academy of Science, Harrisonburg, Virginia, May 4-6, 1966.

2. The structure of dibromoticonine, a bromination produce of nicotine. Herbert McKennis, Jr., Edward R. Bowman, L. D. Quin, and R. C. Denney. 152nd Meeting, American Chemical Society, New York, N. Y., Sept. 12-16, 1966.

3. Some new synthetic routes to metabolites of nicotine. Herbert McKennis, Jr., Biological Seminar, Medical College of Virginia, Richmond, Virginia, Sept. 28, 1966.

4. Quantitative methods for the determination of the distribution of nicotine and its congeners in biological systems. Herbert McKennis, Jr., American Medical Association Workshop, Colorado Springs, Colorado, Nov. 1-3, 1966.

5. Studies on ^{14}C-labelled nicotine metabolites. Edward R. Bowman and Herbert McKennis, Jr., American Medical Association Workshop, Colorado Springs, Colorado, Nov. 1-3, 1966.

6. Pharmacological action and intermediary role of 5-(3-pyridyl)tetrahydrofuranone-2. Edward R. Bowman and Pavel Hrdina. 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

7. Pharmacological effects of some nicotine metabolites and related compounds. K. S. Kim and J. F. Borzelleca, 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

8. Pentafluropropionylation in the determination of nicotine. Herbert McKennis, Jr., S. C. Srivastava, and Edward R. Bowman. Annual Meeting of the Virginia Academy of Science, Roanoke, Virginia, May 6, 1967.

10035469659

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#361R4 GEY

1003546660

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

August 16, 1967

MEMORANDUM

TO: The committee comprising Dr. Little, Chm., Dr. Lynch, Dr. Reimann, Dr. Loosli and Dr. Sommers.

FROM: Robert C. Hockett

SUBJECT: Application for renewal and extension from George O. Gey, M.D. - No. 361-R4.

We enclose herewith a renewal and extension application from Dr. George O. Gey of the Johns Hopkins Medical Institutions of Baltimore, Maryland, together with a comprehensive Progress Report.

Following earlier grants, a five-year plan of research was approved by the Board and activated as of September 1, 1962. It was renewed three times as of the anniversary date. However, as of September 1, 1966, the renewal was made for six months only at Dr. Gey's request (SAB meeting of September 17-18, 1966) to keep the project operating while comprehensive progress reports were prepared. This six month extension to March 1, 1967 left a final six months for the five-year plan.

This comprehensive report of progress has now been received. It contains six parts. Of these, parts four and five seem to the undersigned especially relevant to the current special interests of the Council.

The report is accompanied by an application for continued support for the last six months of the five-year plan, retroactive in effect to March 1, 1967 and also requests a new grant for the period September 1, 1967 to June 30, 1968 in order to conform the anniversary to that of his other major grant. Hence the present application is for sixteen months. The rate per month is, however, unchanged.

This new grant application forecasts two more years at a comparable rate.

While the application proper does not give detail of plans and objectives, these are set forth in context in the six-part progress report.

Council support to Dr. Gey has been long-continued and substantial. We regard this as justifying appointment of large subcommittee for careful evaluation of the rather lengthy reports.

R. C. H.

10035466 61

Source: https://www.industrydocuments.ucsf.edu/docs/pfmh0000

BIOASSAY, CARCINOGENESIS & TISSUE CULTURE

No. 361-R4
Activated: 9/1/62
Renewed: 9/1/63
Renewed: 9/1/64
Renewed: 9/1/65
(All above 1 year)
Renewed: 9/1/66
(6 mo. to 3/1/67)
Cf. #134
Activated: 7/3/56
Renewed annually to 9/1/61

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

COMMITTEE:
Dr. Little, Chm.
Dr. Lynch
Dr. Reimann
Dr. Loosli
Dr. Sommers

633 THIRD AVENUE
NEW YORK, N. Y. 10017

Application For Research Grant

Date: August 4, 1967

1. Name of Investigator(s): (include Title and Degrees) PRINCIPLE INVESTIGATOR: George O. Gey, M.D., Assoc. Prof. Emer. Surg. & Dir. Finney-Howell Can. Res. Lab. CO-INVESTIGATORS: 1)Chiun T. Ling, M.D., Scd., Asst. Prof. Surg. & Phys. Chem.; 2)Mary Reed, Ph.D., Res. Assoc.; 3)Roland Pattillo, M.D., Fellow Surg.; 4)Catherine Armstrong Reznikoff, Ph.D. candidate; 5)Darwin Chee, Grad. Student 6)Tom Depner, Med. Student yr. IV, Res. Assoc.

2. Institution & Address: Finney-Howell Cancer Research Laboratory
Department of Surgery
The Johns Hopkins Medical Institutions
Baltimore, Maryland 21205

3. Short Title of Project: CONTINUATION OF STUDIES ON CHARACTERISTICS OF NORMAL CELL GROWTH IN RELATION TO INVASIVE CARCINOMA, EMBRYONAL TO ADULT FORMS. SUPPORT OF FELLOWSHIPS.

4. Proposed Starting Date: March 1, 1967 (Period March 1-Aug. 31 previously committed). This request to June 30, 1968.

5. Anticipated Duration of this Specific Study:
For the 16-month period given in item 4 with two additional years required.

6. Brief Descripton of Objectives or Specific Aims:

1) Studies on nutrition and environmental factors affecting the growth of cells in culture (Ling & Gey). One prime objective is the evaluation of the nutritional requirements and circumstances under which immunologically competent cells, such as lymphocytes and plasma cells, may be observed to grow and function with the elaboration after challenge of specific antibody.

2) Isolation and studies of normal and malignant lung cells--cytology, karyology, low temperature storage properties, mucous secretion and surfactant production (Reed & Gey).

3) Factors contributing to functional growth of malignant trophoblast. Chemical and biological characterization of chorionic gonadotropin derived in vitro (Pattillo & Gey).

4) Evaluation of cellular transformation in vitro--Role of tumor DNA (Reznikoff & Gey).

5) Evaluation of HeLa cell antigens in a study of genetic exchange of information from host cell to virus (Chee & Gey).

6) Search for immunoglobulins in cultured lymphoblasts of the MBIII (deBruyn-Gey) line (Depner & Gey).

#7 We have already created a number of in vitro cell isolates in attempts to evaluate in vivo cell systems especially in a study of neoplastic processes. Cells in culture permit fundamental studies of growth, differentiation and malignant transformation. As such can also be learned of the functional capacity of normal and malignant cells and may, indeed, provide the best systems for the evaluation of a variety of specific stresses on known histogenic cell types.

7. Give a Brief Statement of your Working Hypothesis:

100354662

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

SEE PROGRESS REPORT.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

SEE ATTACHED SHEET.

10. Additional Requirements:

11. Biographical sketches of all principal and professional personnel (append)

SEE ATTACHED SHEETS.

12. List of publications: (Five most recent as pertinent) (append)

10035466 63

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

Starting & ending when - Loose administration

A. Salaries (Personnel by names)	% time	Amount
Professional		
George O. Gey, M.D., Assoc. Prof. Emer. Surg.	50%	
Chiun T. Ling, M.D., Sc.D., Nutri. Biochem. & Asst. Prof. Surg. (also Asst. Prof. Physiol. Chem.)	50%	
Mary V. Reed, Ph.D., Research Associate	100%	
Technical		
Hedda Linden, Tissue Culture Tech.	100%	
Mary Jo Koryto, Secretary	50%	
Charles Lane, Laboratory Assistant	100%	
Michael Pfeifer, Laboratory & Photog. Asst.	100%	
Franklyn Greene, Laboratory Asst.	100%	
Sub-Total		
(6%) Welfare Benefits		2,412.18
		$42,615.12

B. Consumable Supplies (list by categories)		
Glassware	$764.00	
Chemicals (including photographic)	600.00	
Biologicals (serum, extracts and procurement)	600.00	
Cost of animals and care	300.00	
Photographic film, plates and prints	400.00	
Sub-Total		$2,664.00

C. Other Expenses (itemize)		
Contingency	$ 664.00	
Telephone Rental	1142.10	or $100/mo.
Travel	800.00	
Sub-Total		$2,606.10

D. Permanent Equipment (itemize)		
Equipment — This amount to cover part of purchase of Technicon Sequential Amino Acid Analyzer ($12,340.00). Balance from American Cancer Society and other sources	$3,332.00	$3,332.00

E. Overhead (15% of A+B+C)	7,182.78
Totall	$58,400.00

Estimated Future Requirements: for 16 mo beginning Mar 1967

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

We hope to stress pathogenesis of early tumors such as choriocarcinoma (3 mo. (?) induction period) in comparative normal versus carcinoma cell studies. More travel expenses needed to retrieve specimens. Total cost similar to present basis.

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature _____
Director of Project

Telephone

Signature _____
Business Officer of the Institution

Telephone

10035466 64

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
"Evaluating Factors Contributing to Normal and Malignant Cell Growth" E-273-I	American Cancer Society	$66,835.00	7/1/67 - 6/30/68
Personnel support in collaborative studies with Dr. Milton Edgerton's group on systematic wave length dependent laser studies	W. Alton Jones Foundation, Inc.	$25,000.00 per year	4 years

Pending

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FACILITIES AVAILABLE

The Finney-Howell Cancer Research Laboratory of the Department of Surgery has its facilities established in the Johns Hopkins Hospital. Its facilities include:

(a) An integrated and complete tissue culture laboratory including cubicles accommodating our regular group and pre and post-doctural cancer trainees; also, distilled water autoclave, refrigerator centrifuges, low-temperature refrigerators, special incubators and special observation booths for tissue culture study.

(b) A Cryobiology storage facility including nitrogen refrigerators and programming equipment.

(c) A biochemical laboratory for cell nutrition studies and other chemical studies on chemically-defined media, serum proteins, and tissue culture products.

(d) A motion picture laboratory including:
   1) A shock-proof time lapse motion picture assembly and a special time lapse data recording microscope (Reimer and Gey) for cell population studies.
   2) A 35 mm. automatic film processor.
   3) Dark room facilities.

(e) Electron microscope laboratory (Siemens-Halske unit) complete with dark rooms and thin sectioning equipment.

(f) Adequate animal housing quarters.

(g) Adequate animal operating rooms.

Some of our projects are carried out in collaboration with others as indicated in the statement regarding interdepartmental collaboration. Of particular importance are the opportunities for collaboration with the Department of Pathobiology (Dr. F. B. Bang) on viruses, the Department of Gynecology and Obstetrics in cooperation with Dr. Hugh Davis and others on studies on the development of invasive carcinoma of the cervix. Of special importance is our access to operative specimens from this and many other hospitals and the excellent esprit de corps here and in many remote places in the world allowing rare specimens to be obtained by our former fellows or other friends. The frequent need for choriocarcinoma and other rare specimens requires that the facilities of others be available and in addition to the Tissue Procurement Divisions of the N.C.I. which are also being used.

Our personnel and special facilities have always been available to others in this and other institutions for conferences and especially for their benefit in allowing procurement of continuous cultures of many different cell types.

10035466666

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Gey, George Otto, B.S., M.D..

R R R

Present Address: Finney-Howell Cancer Research Laboratory, 13th Floor, RU #1 The Johns Hopkins Hospital, Baltimore 5, Md., 1933.

SCIENTIFIC AND ACADEMIC POSITIONS:

1921 - Carnegie Research Assistant, Cold Springs Harbor, L.I., N.Y..

1921-22 - Instructor in Zoology, University of Pittsburgh.

1922-23 - Guest Research Student, Dept. of Embryology, Carnegie Inst., Baltimore, Md..

1923-29 - Cancer Research Fellow, Columbia Hospital, Milwaukee, Wisc..

1929-33 - Director, Tissue Culture Laboratory, Dept. of Surgery, and Student Assistant in Surgery, Johns Hopkins Medical School.

1929-39 - Guest Research Associate, Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, Md..

1933-38 - Assistant in Surgery, Johns Hopkins Medical School.

1933-36 - Surgeon (R), U.S.P.H.S., National Cancer Institute. Director, Cancer Research - Co-Operative Studies, U.S.P.H.S. and Johns Hopkins Medical School, Baltimore, Md..

1940-41 - Finney-Howell Cancer Research Fellow.

1941-45 - Commander, Emergency Medical Services, Office of Civilian Defense, Eastern District, Baltimore, Md..

1938-46 - Instructor in Surgery, Johns Hopkins Medical School.

1940-46 - Assistant Director, Division of Cell Physiology, Dept. of Surgery, Johns Hopkins Medical School.

1947- - - Assistant Professor of Surgery, Johns Hopkins Medical School.

1947- - - Director, Finney-Howell Cancer Research Laboratory, Department of Surgery, The Johns Hopkins Medical School and Hospital.

1954 - Lectr. Pathobiology, School of Hygiene and Public Health, The Johns Hopkins Medical School.

1003546667

1958 - Associate Prof. Surg., The Johns Hopkins Medical School.

1966 - Associate Prof. Surg. (Emeritus) Active Duty, The Johns Hopkins Medical School.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS:

Gey, George O. (with Lewis, W.H.): Clasmatocytes and Tumor Cells in Cultures of Mouse Sarcoma, Bull. Johns Hopkins Hosp., 34:369, 1923.

_____ and Thalhimer, W. 1924. Observations on the Effects of Insulin Introduced into the Medium of Tissue Cultures, J.A.M.A., 82:1609.

_____ with Cron, R.S. 1927. The Viability of the Cast-Off Menstrual Endometrium, Am. J. Obs. & Gyn. 13:645.

_____ Studies on the Cultivation of Human Tissue Outside the Body. 1929. Wisconsin J. J. 28:11.

_____ An Improved Technic for Massive Tissue Cultures. 1933. Am. J. Cancer 17:752.

_____ with Stone, H.B., and Owings, J.C. 1933. Living Grafts of Endocrine Glands, California and Western Medicine, 38: No. 6; 39: Nos. 1 and 2, June, July and August, 1933.

_____ with Stone, H.B., and Owings, J.C., Living Grafts of Endocrine Glands, Am. Jour. Surgery, New Series, 24:1934.

_____ with Stone, H.B., and Owings, J.C. 1934. Transplantation of Living Grafts of Thyroid and Parathyroid Glands. Annals of Surg. 262.

_____ with Stone, H.B., and Owings, J.C. Feb. 1935. Living Grafts of Thyroid and Parathyroid Glands. Surg. Gyn. Obs..

_____ and M.K. Gey. The Maintenance of Human Normal Cells and Human Tumor Cells in Continuous Culture. 1. Preliminary Report. Cultivation of Mesoblastic Tumors and Normal Tissue and Notes on Methods of Cultivation. Am. J. Cancer 27:45. May 1936.

_____ with Seegar, G.E., and Hellman, L.M. The Production of a Gonadotrophic Substance (Prolan) by Placental Cells in Tissue Culture. Sci. 88:306 1938.

_____ with Stone, H.B., and Owings, J.C. 1938. Further Reports on Grafting of Endocrine Glands. Mis-South Post-Graduate Medical Assembly, Memphis, Tenn. Feb. 16, 1938.

_____ and Bang, F.B.. Experimental Studies on the Cultural Behavior and the Infectivity of Lymphopathia Venerea Virus Maintained in Tissue Culture. Bull. Johns Hopkins Hosp., 45: No.5, Nov. 1939.

_____ with Gemmill, C.L., and Austrian, R. The Metabolism of Tissue Cultures of Walker Rat Sarcoma 319, Bull. Johns Hopkins Hosp., 66: 167-184, 1940.

1003546668

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS (CONT'D)

Gey, George O., Cytological and Cultural Observations on Transplantable Rat Sarcomata Produced by the Innoculation of Altered Normal Cells Maintained in Continuous Culture. Cancer Research 1:737, 1941.

_____ with Seager Jones, G.E., and Gey, M.K. 1943. Hormone Production by Placental Cells Maintained in Continuous Culture. Bull. Johns Hopkins Hosp. 72:26.

_____ and Firor, W.M. 1945. Observations on the Conversion of Normal Into Malignant Cells. Annals of Sur., 121.

_____ with Gey, M.K., Inui, F. and Vedder, H. 1944. Penicillin Action on Strains of Normal and Cancer Cells. A.A.A.S. Res. Conf. on Cancer, 321.

_____ Gey, M.K., Inui, F., and Vedder, H. The Effects of Crude and Purified Penicillin on Continuous Cultures of Normal and Malignant cells. Bull. Johns Hopkins Hosp. 77:116-131, 1945.

_____ with Firor, W.M., Phase Contrast Microscopy of Living Cells. Annals of Sur., 125:604.

_____ Hanks, J.H., and Barrett, Rachel. 1948. Retardation of Growth and Metabolism of Normal and Malignant Cells During Continuous Cultivation. Growth 12:69.

_____ with Bang, F.B. 1948. A Fibrillar Structure in Rat Fibroblasts as seen by Electron Microscopy. Proc. Soc. Exptl. Biol. Med. 69:86.

_____ with Bang, F.B. 1949. Electron Microscopy of Tissue Cultures Infected with the Virus of Eastern Equine Encephalomyelitis. Proc. Soc. Exptl. Biol. Med. 71:78.

_____ Gey, M.K., Firor, W.M., and Self, W.O. 1949. Cultural and Cytologic Studies on Autologous Normal and Malignant Cells of Specific in vitro Origin. Acta. Un. Int. Con. Cancrum. 6:706.

_____ and Bang, F.B. 1951. Viruses and Cells. A Study in Tissue Culture Applications. I. Cells Involved--Availability and Susceptibility. Trans. N.Y. Acad. Sci. 14:15.

_____ with Bang, F.B. 1951. Viruses and Cells. A Study in Tissue Culture Applications. II. Effect of Several Viruses on Cell Types and the Amount of Virus Produced. Trans. N.Y. Acad. Sci. 13:324.

_____ with Bang, F.B. and Levy, E. 1951. Some Observations on Host-Cell Virus Relationships in Fowl Pox. I. Growth in Tissue Culture. II. The Inclusion Produced by the Virus on the Chick Chorio-Allantoic Membrane. J. Immun. 66:329.

1003546669

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS (CONT'D)

Gey, George O., with Bang, F.B. 1952. Comparative Susceptibility of Cultured Cell Strains to the Virus of Eastern Equine Encephalomyelitis. Bull. Johns Hopkins Hosp. 91:427.

_____ with Glinos, Andre D. 1952. Humoral Factors Involved in the Induction of Liver Regeneration in the Rat. Proc. Soc. Exptl. Biol. Med. 80:421.

_____ with Shooter, R.A. 1952. Studies on the Mineral Requirements of Mammalian Cells. Brit. J. Exptl. Path. 33:98.

_____ 1952. Proceedings of the Research Session on Endocrinologic Aspects of Aging. Edited by V. Korenchevsky. J. Gerontology. 7.

_____ 1953. Discussion in "Problems of Aging" edited by Nathan W. Stock, from 15th Macy Conference.

_____ with Ehrmann, R.L. 1953. The Use of Cell Colonies on Glass for Evaluating Nutrition and Growth in Roller-Tube Cultures. J. Natl. Cancer Inst. 13:1099.

_____ with Scherer, W.F., and Syverton, J.T. 1953. Studies on the Propagation in vitro of Poliomyelitis Viruses. IV. Viral Multiplication in a Stable Strain of Human Malignant Epithelial Cells (strain hela) derived from an Epidermoid Carcinoma of the Cervix. J. Exptl. Med. 97:695.

_____ 1954-55. Some Aspects of the Constitution and Behavior of Normal and Malignant Cells Maintained in Continuous Culture. The Harvey Lectures, Series L. Academic Press Inc., N.Y. 154.

_____ Bang, F.B., and Gey, M.K. 1954. Responses of a Variety of Normal and Malignant Cells to Continuous Cultivation and Some Practical Applications of these Responses to Problems in the Biology Disease. Ann. N.Y. Acad. Sci. 58:976.

_____ with Owens, O.v.H., and Gey, M.K. 1954. Growth of Cells in Agitated Fluid Medium. Ann. N.Y. Acad. Sci., 58:1039-1055.

_____ Shapras, P., and Borysko, E. 1954. Activities and Responses of Living Cells and their Components as Recorded by Cinephase Microscopy and Electron Microscopy. Ann. N.Y. Acad. Sci. 58:1089.

_____ Bang, F.B., and Gey, M.K. 1954. An Evaluation of Some Comparative Studies on Cultured Strains of Normal and Malignant Cells of Animals and Man. Texas Reports. Biol. Med. 12:805.

1003546670

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS (CONT'D)

Gey, George O., with Lo, W.H.Y., Bang, F.B. and Shapras, P. 1955. Rous Sarcoma Virus Infections in the Chick Embryo I and II and The Cytopathogenic Effect of the Rous Sarcoma Virus on Chicken Fibroblasts in Tissue Cultures. Bull. Johns Hopkins Hosp. 97:227.

_____ Shapras, P., Bang, F.B. and Gey, M.K. 1955. Some Relations of Inclusion Droplets (pinocytosis-Lewis) and Mitochondrial Behavior in Normal and Malignant Cells. Symposium on Fine Structure of Cells. E.P. Noordhoff, Ltd., Groningen, 38.

_____ with R. L. Ehrmann, 1956. The Growth of Cells on a Transparent Gel of Reconstituted Rat-Tail Collagen. J. Natl. Cancer Inst. 16:1375.

_____ Discussion, Tissue Culture in the Study of Animal and Human Tumors. 1957. 19:784-785, J. Nat. Cancer Inst.

_____ Factors Influencing Proliferation of Viruses in Normal and Malignant Cells. 1957. Texas Reports No. 3 534-539.

_____ with F. B. Bang, M. Ford and D. Minnegan, 1957. Chronic Infections Produced in Cultured Cell Strains by the Virus of Eastern Equine Encephalomyelitis. Acad. Press Inc., Virology, 4, No. 3.

_____ with H. N. Kent, 1960. Selective Uptake of Serum Globulins and Glycoproteins by Cells Growing in Vitro. Science, 131:666-68.

_____ with M. V. Reed, 1962. Cultivation of Normal and Malignant Human Lung Tissue. Lab. Investigations, 11:8, pp. 638-52.

_____ with J. K. Frost, 1962. Symposium: Cell Modulations, Maturation, and Neoplastic Transformation. Acta Cytologica, 6:399-402.

_____ with C. T. Ling and V. Richters. Chemically Characterized Concentrated Corodice for Continuous Cell Culture (7 C's Solution). Paper presented at the Tissue Culture Association Meeting, Washington, D.C., May 31, 1962.

_____ with R. A. Milch and M. A. Naughton, 1965. Submit Patterns of Rapidly Proliferating Connective Tissues. Biochem. Biophys. Acta 100:623-26.

_____ with M. K. Gey, M. Svotelis, and H. Linden. A Motion Picture Sequence Illustrating the Production of Giant Cells from Hypodiploid Rat Strains NSAT-14pH and -14pL. Presented at the 16th Annual Meeting of the Tissue Culture Association, May 31-June 3, 1965.

1003546671

_____ with C. T. Ling. The Role and Interaction of Trace Elements in Cell Nutrition. Presented at the 17th Annual Meeting of the Tissue Culture Association, May 31-June 3, 1966.

_____ with C. T. Ling. Further Studies on the Role and Interaction of Trace Elements in Cell Nutrition. Presented at the 18th Annual Meeting of the Tissue Culture Association, June 4-7, 1967.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

SCIENTIFIC ORGANIZATIONS

REDACTED

REDACTED

REDACTED

REDACTED

REDACTED

PROFESSIONAL CLUBS

REDACTED

INVESTIGATOR AND GRANTEE

Frances P. Garvan Cancer Fund, Chemical Foundation 1929-31.
The International Cancer Research Foundation 1932-37.
National Cancer Institute 1948.
American Cancer Society 1949.
Medical Consultant and Grantee, Committee on Hemorrhagic Fever, Armed Forces Epidemiology Board 1953.
National Foundation for Infantile Paralysis 1953-55.

AWARDS

Judd Award Cancer Research 1954.
Wein Award Cancer Cytology 1956.

10035466/2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Movie making must be expensive

SCIENTIFIC RESEARCH FILMS RELEASED:

Gey, G. O., and Lewis, W.H., THE CULTIVATION OF HUMAN TUMORS IN VITRO, Garvan Cancer Research Foundation, Chem. Foundation Inc., N.Y., 1931.

_____, EFFECTS OF IRRADIATION ON TISSUE CULTURES, Eastern Conference of Radiologist, 1936.

_____, Gey, M.K., Stone, H.B., and Owings, J.C., TISSUE CULTURES OF ENDOCRINE ORGANS. American Association of Anatomists, 1938.

_____, Lewis, M.R., Gey, M.K., CULTIVATION OF BLOOD CELL TUMORS OF THE MOUSE. American Association for Cancer Research, 1938.

_____, with Firor, W.M., OBSERVATION ON RAT TUMOR CELLS FROM NORMAL CELLS IN VITRO. American Cancer Society, N.Y., 1945.

_____, with Firor, W.M., and Self, W.O., CYTOLOGICAL BEHAVIOR OF AUTOLOGOUS NORMAL AND MALIGNANT CELLS, A Study with phase contrast microscopy, Int. Cancer Congress, 1946.

_____, Shapras, P., and Bang, F.B., NORMAL AND MALIGNANT CELLS,

(a) STUDIES OF NORMAL AND MALIGNANT RAT CELLS OF ISOGENIC ORIGIN, Phase and Electron Microscope Observations.

(b) OBSERVATIONS ON HUMAN CANCER CELLS, Strains D-1 Re; A. Fi; and HeLa, CINE-PHASE AND ELECTRON MICROSCOPE. STUDIES ON MEMBRANE ACTIVITY AND MITOCHONDRIAL BEHAVIOR, American Cancer Society, 1946-1955.

_____, Shapras, P., Bang, F.B., and Pazemenas, K., ACTIVITY OF MITODIONDRIA AND PLASMA MEMBRANE IN NORMAL AND MALIGNANT CELLS RAT AND HUMAN, American Cancer Society, 1946-1957.

_____, Gey, M. K., Svotelis, M., and Linden, H., A Motion Picture Sequence Illustrating the Production of Giant Cells from Hypodiploid Rat Strains NSAT-14pH and -14pL. Presented at the 16th Annual Meeting of the Tissue Culture Association, May 31-June 3, 1965.

10035466 73

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

NAME: Chiun T. Ling

R R R REDACTED

LANGUAGES: English, German, French, Japanese, Chinese.

STUDIES AND DEGREES:

Tung Wen College, Fukien, China (Premedical), 1919-21

Nippon Medical College, Tokyo, Japan (Premedical), 1921-22

Nippon Medical College, Tokyo, Japan (Medical), 1922-26
Degree: M.D., 1926

REDACTED

Johns Hopkins University, Baltimore, Maryland, 1945-46
Degree: M.P.H., 1946

Harvard University, Boston, Massachusetts, 1946-47 (Research in Nutrition)

Johns Hopkins University, Baltimore, Maryland, 1948-52
Degree: Sc.D. (Biochemistry), 1952

Internship: University Hospital, Tokyo, Japan, 1926-27

Assistant Resident: University Hospital, Tokyo, Japan, 1927-29

Resident in surgery: Red Cross Hospital, Tokyo, Japan, 1929-30

ACADEMIC APPOINTMENTS:

Research Associate in Biochemistry, Johns Hopkins University, 1952-53

Assistant Professor of Biochemistry, Jefferson Medical College, 1953-57

Assistant Professor of Biological Chemistry, University of Michigan Medical School, 1957-59

Research Associate, Simpson Memorial Institute, University of Michigan, 1957-59

Instructor in Surgery, Johns Hopkins University, School of Medicine, 1959-62

Instructor in Physiological Chemistry, Johns Hopkins University, School of Medicine, 1959-62

Asst. Prof. Surgery; Asst. Prof. Physiological Chemistry, Johns Hopkins University School of Medicine, 1962-to date

EXPERIENCE:

Over 15 years of experience in biochemical and nutritional research. The following outlines the nature of problems studied and the techniques used.

10035469674

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Curriculum Vitae - Dr. Chiun T. Ling

Animal experimentation and in vitro studies with tissue materials, on the functions of vitamins and their effects on carbohydrate, protein, and lipid metabolism.

Quantitative micro techniques for the analyses of carbohydrates, proteins, lipids and nucleic acids, coenzyme assays, etc.

Chromatographic and electrophoretic analyses.

Radioisotope work with Co^{60}, Fe^{59}, and C^{14}.

Isolation of leucocytes and determination of thymidine contents in normal and leukemic cells.

Isolation of erythrocyte stromata and studying their chemical composition in normal and pathologic cells.

Five years of experience in teaching medical biochemistry, consisting of:

a) Formal illustrated lectures 17 - 18 hours each year
b) Supervision of group experiments by medical students
c) Supervision of laboratory work, group conferences and students counseling
d) Supervision of graduate students

PREVIOUS RESEARCH ACTIVITIES:

Studies on the formation of lipid components of blood cells in bone marrow; incorporation of labeled precursors into components of globoside, phospholipids, and sterol of the cells in vitro with bone marrow from normal, anemic and leukemic animals; changes in enzyme and coenzyme activities; effects of vitamin antagonists in these systems.

PRESENT RESEARCH ACTIVITIES:

1. Studies on the Nutrition of Normal and Malignant Cells in Culture.

A concentrated chemically characterized medium has been developed which maintains MBIII lymphoblasts and Walker Rat Carcino-sarcoma-256 cells in continuous culture. Investigations in progress include:

a) The influence of intracellular amino acid concentration on the formation of cell proteins and its dependence on amino acid levels in culture media.

b) Studies on the quantitative evaluation of cell growth in vitro; interrelationships between cell volume, cell count, cell protein and nucleic acid contents (DNA/RNA ratio).

c) Effect of the nature of glass walls of culture vessels and their chemical treatment on cell survival, growth and behavior.

10035466675

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Curriculum Vitae - Dr. Chiun T. Ling

2. Lipid Metabolism of Cells in Culture

Investigations on the Metabolic Interaction between Cholesterol, Phospholipids and Fatty Acids.

3. Other Research Interests

Nutritional requirements of normal bone marrow cells in culture and factors affecting cell differentiation.

Culture requirement of leukemic cells

Immunochemical reactions between tissue cells in vitro

TEACHING ACTIVITIES:

Teaching and conducting experiments with students in nutritional biochemistry as part of the biochemistry course given during one quarter only to second year medical students by the Department of Physiological Chemistry. Students' work includes studies of changes in tissue enzymic activities in nutritional deficiency. Reference - Laboratory Manual for Biochemistry, Section VIII, Dept. of Physiological Chemistry.

1003546676

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LIST OF PUBLICATIONS AND REPORTS - Dr. Chiun T. Ling

Ling, C. T., Hegsted, D. M., and Stare, F. J.: The Effect of Pyridoxin Deficiency on the Niacin-Tryptophane Transformation in Rats. J. Biol. Chem., 174:803, (1948).

Chow, B. F., Barrows, L., and Ling, C. T.: The Distribution of Radioactivity in the Organs of the Fetus or of the Young Rats Born of Mothers Injected with B_{12} containing Co^{60} Arch. of Biochem. & Biophys., 34:151, (1951).

Ling, C. T., and Chow, B. F.: The Effect of Vitamin B_{12} on the Body Composition of Rats. J. Biol. Chem., 198:439, (1952).

Ling, C. T., and Chow, B. F.: The Effect of Vitamin B_{12} on the Levels of Soluble Sulfhydryl Compounds in Blood. J. Biol. Chem., 202:445, (1953).

Ling, C. T., and Chow, B. F.: The Influence of Vitamin B_{12} on Carbohydrate and Lipid Metabolism. J. Biol. Chem., 206:797, (1954).

Ling, C. T., and Chow, B. F.: The Effect of Vitamin B_{12} on Ribose Formation by Erythrocytes. Federation Proc., 13:253, (1954).

Ling, C. T., and Chow, B. F.: Vitamin B_{12} and Carbohydrate Metabolism in First European Symposium on Vitamin B_{12} and Intrinsic Factor. Ferdinand Enke Verlag, Stuttgart, Germany (1956).

Ling, C. T.: Vitamin B_{12} and Lipid Metabolism. Abstracts of Papers, Am. Chem. Soc. 134th National Meetings, 57C, (1958).

Ling, Chiun T.: Role of Vitamin B_{12} in Lipid Metabolism. Federation Proc. 18:489, (1959).

Ling, Chiun T., Gey, G. O., and Richters, V.: Seven Cs Solutions: Chemically Characterized Concentrated Corodice for Continuous Cell Culture. Submitted for presentation to the 53rd Annual Meeting of the American Association for Cancer Research, April, 1962.

Ling, C. T., and Gey, G. O.: The Role and Interaction of Trace Elements in Cell Nutrition. Submitted for presentation to the 17th Annual Meeting of the Tissue Culture Association, May 31-June 3, 1966.

Ling, C. T., and Gey, G. O.: Further Studies on the Role and Interaction of Trace Elements in Cell Nutrition. Submitted for presentation to the 18th Annual Meeting of the Tissue Culture Association, June 4-7, 1967.

Copy of paper submitted with application. To be published soon.

10035469472

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Mary V. Reed

R REDACTED REDACTED REDACTED

Address:

(Business) 601 N. Broadway, Baltimore, Maryland 21205

Education:

	Dates	Degree	Date of Degree
Goucher College	R	A.B.	June, 1931
Smith College		A.M.	June, 1933
Johns Hopkins University School of Hygiene & Public Health		Special Student	-------
University of Maryland (Medical School)	R R	Ph.D.	January, 1954

Master's Thesis - The effect of certain triphenylmethane dyes on Staphylococcus aureus and Escherichia coli communior

Doctoral dissertation - A comparative study of the metabolism of a chloramphenicol-sensitive strain and a chloramphenicol-resistant strain of Escherichia coli

Professional Society Memberships

REDACTED REDACTED

Academic Honors - Sigma Xi - Johns Hopkins University

Teaching Positions

Graduate Assistant in Bacteriology, Smith College, Northampton, Massachusetts, 1931-1933.

Assistant in Bacteriology, Smith College, 1933-1934.

Instructor in Science and Mathematics, The Knox School, Cooperstown, New York, 1934-39, 1945-46.

Instructor In Science, Mount Vernon Seminary & Junior College, Washington, D.C., 1939-45.

Assistant Professor in Chemistry and Nutrition, University of Tennessee, Knoxville, Tennessee, 1946-48.

10035466/8

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Curriculum Vitae - Dr. Mary V. Reed

Instructor in Physiology and Bacteriology, Goucher College, Baltimore, Maryland, 1949-1954, Assistant Professor, 1954-55.

Postdoctoral Fellow, The Johns Hopkins Medical School, Baltimore, Maryland, 1955-1965.

Research Associate, The Johns Hopkins Medical School, Baltimore, Maryland, 1965-to date.

Publications

Reed, M. V., and Genung, E. F.: The Effect of Certain Triphenylmethane dyes on Staphylococcus aureus and Escherichia coli communior. J. Bact., 27, 29, (1934).

Reed, M. V., and Genung, E. F.: Titration of Dyes for their Bacteriostatic Action. Stain. Tech., 9, 117-128, (1934).

Reed, M.V., and Gey, G. O.: Cultivation of Normal and Malignant Human Lung Tissue. 1. The establishment of three adenocarcinoma cell strains. Lab. Investigations, 11, 638-652, (1962).

10035466679

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

ROLAND ANTHONY PATTILLO

R

REDACTED REDACTED REDACTED

Address:

(Business) 601 N. Broadway, Baltimore, Maryland

Education: Blessed Sacrament High School, Beaumont, Texas 1951

B.S. Xavier University, New Orleans, Louisiana 1955

M.D. St. Louis University School of Medicine 1959 R R

Internship: Milwaukee County General Hospital 1959-1960
Milwaukee, Wisconsin

Residency: Obstetrics & Gynecology

Under Dr. Richard F. Mattingly, Professor & Chairman,
and Dr. Eleanor Delfs, Professor of Obstetrics,
Department of Gynecology & Obstetrics
Marquette University School of Medicine

Inclusive of Exchange Resident rotations:

Boston Lying-In Hospital, under Dr. Duncan Reid,
Professor & Chairman, Department of Obstetrics & Gynecology,
Harvard University, Boston, Massachusetts

General Surgery & Urology, under Dr. Edwin Ellison's
direction, Professor and Chairman, Department of Surgery,
Marquette University School of Medicine

General, Gynecologic & Obstetric Pathology, under
Dr. Paul Kimmelstiel, Research Professor of Pathology,
Marquette University School of Medicine

Academic: Instructor, Department of Gynecology & Obstetrics 1964 -
Marquette University School of Medicine

Research Fellow, National Institute of Health 1964 -

Fellow in Surgery, The Johns Hopkins University 1966 -

Hospital Appointments:

Staff: St. Joseph's Hospital; Mt. Sinai Hospital
St. Michael Hospital, Milwaukee, Wisconsin

Attending Staff: Milwaukee County General Hospital

10035466680

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Curriculum Vitae - Dr. Roland A. Pattillo

Family:

REDACTED REDACTED REDACTED

Professional Associations:

REDACTED REDACTED REDACTED

Bibliography:

Pattillo, R. A., Foley, D. V., and Mattingly, R. F.: Internal Pelvic Lymphography. American Journal of Obstetrics & Gynecology, 88:110, (1964).

Pattillo, R. A. and San Felippa, P. M.: Carcinoma of the Vulva. Marquette Medical Review, 31:121, (May, 1965).

Pattillo, R. A., Smith, T. E., Delfs, E., and Mattingly, R. F.: Cytologic and Hormonal Activity of the Trophoblast in Explant Culture. American Journal of Obstetrics and Gynecology, 96:337, (1966).

Pattillo, R. A.: The Human Trophoblast--Its Endocrine and Neoplastic Properties. Marquette Medical Review, 32, No. 4, (1966).

Pattillo, R. A., Mattingly, R. F. and Messinger, P. S.: Visicocervicorectal Fistula: A New Gynecologic Entity. Obstetrics & Gynecology, 27:432, (1966).

Continuing Investigation:

Carcinogenic Stimulation of the Rat Placenta - Attempts are being made to create an animal model for choriocarcinoma. The carcinogen 7,12 dimethylbenzanthracene is deposited at the gestational site in early and mid pregnancy in Hooded rats. Hormonal support in the form of estrogen and progesterone is supplied exogenously in order to maintain placental viability. Evidence for neoplastic changes is being sought.

Reactions of the Human Trophoblast as Explant and Transplant- Human trophoblastic tissue obtained sterilely from ectopic gestations and sterile mature placentas from Cesarean sections are propagated in roller tube, T-Flask and organ cultures. Studies are directed toward identifying the cell of origin of chorionic gonadotropin and steroid hormones. Comparisons of the differences in behavior of hydatiform mole and choriocarcinoma as to functional and cytologic characteristics are being sought.

Immunologic factors in normal trophoblast and their variants and similarities in trophoblastic neoplasms are being evaluated by transplantation to thymectomized neonatal rats.

10035469681

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#409R3 BANG

1003546682

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

August 23, 1967

MEMORANDUM

TO: The committee comprising Dr. Reimann, Chm., Dr. Sommers and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Frederik B. Bang, M.D. - No. 409-R3.

We enclose herewith a renewal application from Dr. Frederick B. Bang, of the Johns Hopkins University, Baltimore, Maryland.

This request represents the fourth year of an original five year plan. At the time of the last renewal prior consideration was promised for a further two years in the allocation of available funds. However, it should be noted that the anticipated duration of the study is now given as three years which would extend the total duration to six years.

R. C. H.

1003546683

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

VIRUS, PREVIOUS INFECTIONS

#409R3
Activated: 10/1/64
Renewed: 10/1/65
Renewed: 10/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Reimann, Chm.
Dr. Sommers
Dr. Jacobson

Application For Research Grant

Date: July 20, 1967

1. Name of Investigator(s): (include Title and Degrees)
Frederik B. Bang, M.D., Professor of Pathobiology

2. Institution & Address:
The Johns Hopkins University School of Hygiene and Public Health
615 North Wolfe Street, Baltimore, Maryland - 21205

3. Short Title of Project:
Pathogenesis of virus infections of respiratory mucosae

4. Proposed Starting Date:
To be continuous with present grant

5. Anticipated Duration of this Specific Study:
Three years

6. Brief Descripton of Objectives or Specific Aims:

1) Continue to study the effects of drugs and of internal and external environmental factors on susceptibility to viral infections, with emphasis on the initiation of infection.

2) Initiate a sequential study of regeneration and re-organization of mucosal cells following acute desquamation; for this study, nuclei will be labelled with tritiated thymidine, a method used extensively in studies of intestinal mucosae.

3) Study the effects of avitaminosis A on the susceptibility of chickens to the viruses of Newcastle disease and laryngotracheitis; we have found in preliminary studies that avitaminosis A (known to affect mucous epithelia) shows the first apparent effects histologically on the inner surface of the scroll of the maxillary concha, an area which we have found eminently vulnerable to virus infection.

4) With the collaboration of Dr. Donald F. Proctor, we propose to determine the effects of acute upper respiratory infections on the rate of nasal mucus clearance before, during, and after the acute clinical phase of infection; this will be a study on human volunteers, using a method which has proved reliable in a recent study which involved several hundred tests.

(Items 1, 3, and 4 will extend studies which have been primarily supported by the Council for Tobacco Research, and which have been published or are in press, as noted under item 12 below)

7. Give a Brief Statement of your Working Hypothesis:

Items 7, 11, 12 are on a separate attached page

100354684

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

1: Drugs are administered to chicks intramuscularly or locally; NDV is inoculated intranasally. Chicks are killed at intervals, the maxillary conchae excised, extensively washed, scraped, and the cells trypsinized; the number of infected cells is then determined by plaque techniques. 2: Tritiated thymidine labelling will be done in pulses, and the progress and persistence of labelled cells determined at successive time intervals. 3: Chickens fed for 3 weeks after hatching on a commercial vitamin A-deprived diet will be inoculated with the two viruses and the spread of virus in the respiratory tract will be followed by titrations and by histology. 4: The rate of mucociliary clearance of the upper respiratory tracts of human volunteers will be recorded before, during, and after spontaneous acute upper tract infection. This is done by injecting a chemically inert tracer dye (Direct Sky Blue, Wyeth) onto a particular site on the anterior mucociliated part of the nasal fossa and timing its appearance in the nasopharynx, a method pre-tested for accuracy by running it simultaneously with a radioactive tracer in a series of volunteers.

(The numbers above are consistent with those of the experimental objectives given in Item 6)

9. Physical Facilties Available (Where Other than Administering Organization Indicate Geographical Location)

Fully equipped virus and histological laboratories.

10. Additional Requirements:

1) A full time histological technical trainee for routine histological sectioning; the present technical assistant, Mr. Raoul Spicker, to train this apprentice and to devote most of his own efforts toward autoradiography.

2) Half-time support for an additional animal man, to cover the increased use of animals requiring reliable supervision.

11. Biographical sketches of all principal and professional personnel (append)

(appended)

12. List of publications: (Five most recent as pertinent) (append) (appended)

10035466B5

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Working hypothesis

1) Alterations in the mucous-ciliated epithelium which are induced by changes in the external and/or internal environment of the host will affect viral entry into mucosal cells.

2) The progress of infection from cell to cell, and the recovery of the mucous-ciliated epithelium following infection, are affected by mucous secretion. Avitaminosis A, known to induce conversion of mucous epithelium to squamous epithelium, should alter the progress of infection, particularly myxovirus infection

11. Biographical sketches

Frederik B. Bang, M.D., - b. November 5, 1916 in Philadelphia Pennsylvania. A.B. Johns Hopkins University 1935; M.D. Johns Hopkins School of Medicine 1939. Associate Professor of Medicine, Johns Hopkins School of Medicine 1949-1953; Professor of Pathobiology, Johns Hopkins School of Hygiene and Public Health 1953-1967. Special interests: virus pathogenesis; invertebrate immunology.

Betsy G. Bang, A.B., - b. July 9, 1912 in Lancaster, South Carolina. A.B. George Washington University 1933; Certification in Art as Applied to Medicine, Johns Hopkins School of Medicine 1937. Illustrator in Comparative Anatomy, American Museum Natural History 1937 - 1940; Research Associate in Pathobiology, Johns Hopkins School of Hygiene and Public Health 1962-1967. Specific interest: comparative functional anatomy of the upper respiratory tract.

Prantika Som, D.V.M., - b. August 31, 1942, Silchar, India. Inter-Science, Lady Bradbourne College, University of Calcutta 1960; B.V.Sc. and A.H., and D.V.M. Bengal Veterinary College, University of Calcutta 1965. Candidate for Sc.D. degree in Department of Pathobiology, Johns Hopkins University School of Hygiene and Public Health.

12. Recent pertinent publications

(1) Bang, F. B., and Foard, M.: Interaction of respiratory epithelium of the chick and Newcastle disease virus. Amer. J. Hygiene 1964 79:260

(2) Huang, J., and Bang, F.B.: The susceptibility of chick embryo skin organ cultures to influenza virus following excess vitamin A J. Exp. Med. 1964 120:129-148

(3) Bang, F. B., Bang, B. G., and Foard, M.: Responses of upper respiratory mucosae to drugs and viral infections. Amer. Review of Resp. Dis. 1966 93:142-149

(4) Bang, B.G., and Bang, F.B.: Laryngotracheitis virus of chickens: a model for acute desquamating rhinitis. J. Exp. Med. 1967 125:409-428

(5) Bang, B.G., Mukherjee, A.L., and Bang, F.B.: Human nasal mucus flow rates. (In press: Johns Hopkins Medical Journal) (In press: simultaneous abstract in J. Amer. Med. Assoc.)

10035466686

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

A. Salaries (Personnel by names) Professional		% time	Amount
Marie A. Foard	Research Asst. in Pathobiology	50%	$3625.00
TECHNICAL			
Raoul Spicker	Histologist	50%	3000.00
To be named	Histological Trainee	100%	5000.00
Technical Henry Johnson	Animal Attendant	88%	3060.00
Juhan Labidas	Animal Attendant	50%	1500.00
Linda Endemann	Glassware Washer	100%	3645.00
Gwendolyn Futcher	Secretary	12%	752.00
		Sub-Total	$20582.00
B. Consumable Supplies (list by categories)			
Animal Food			1000.00
Histological Material			1000.00
Publications			600.00
		Sub-Total	$2600.00
C. Other Expenses (itemize)			
Travel-Domestic-to attend clinics			800.00
Requisition Payroll-to cover temporary summer help, termination payroll and special services			500.00
Fringe Benefits-6%-salaries and wages			1265.00
		Sub-Total	$2565.00
D. Permanent Equipment (itemize)			
Animal cages			1000.00
			$26747.00
E. Overhead (15% of A+B+C)			3862.00
		Total	$30609.00

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	$21318.00	$2600.00	$2610.00	$1000.00	$3980.00	$31508.00
Year 3	$23045.00	$2600.00	$2713.00	$1000.00	$4254.00	$33612.00

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature _____
Director of Project

Telephone

Signature _____
Business Officer of the Institution

Telephone

10035466B7

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Comparative Anatomy of the Vertebrate Nasal Organ	NSF #GB5315	$12800.00	7/1/66-6/30/69

Pending

None

This grant to B.G. Bang finances a study of the comparative functional anatomy of the nasal folfa, and is the source of ½ of Mr. Spicker's salary.

1003546688

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#455R1 ROSE

1003546689

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

September 5, 1967

MEMORANDUM

TO: The committee comprising Dr. Sommers, Chairman, Dr. Cattell and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: A renewal application from Charles L. Rose, A.M. and Benjamin Bell, M.D. - #455R1.

On April 1, 1965, the Scientific Advisory Board awarded a $10,000 "piggy-back" grant to Mr. Charles Rose and Dr. Benjamin Bell of the Boston V.A. Outpatient Clinic for assisting a retrospective longevity study. On August 1, 1967, with the approval of the committee indicated, an additional $3,300 was given as a supplement to carry personnel through to October 1, 1967. This new proposal will carry forward the "seeding" concept initiated by this group.

The unusual husbanding of funds along eighteen months beyond the original termination date is one of the minor recommendations for this award.

The major recommendations evolve around the fact that these data plus the new Normative Aging Studies, prospective, serve as the keystone to the staff project being carried out by Kurt Enslein dealing with the development of computer methods for analyzing multivariate data. He has successfully developed methods to handle in excess of 100 variables simultaneously.

It has already become evident that smoking is a result of a number of other characteristics and does not bear a primary relationship to longevity.

As indicated in the enclosed progress reports, your support to this group is reaping the benefit of $154,000 per year of V.A. funding and at least an equal amount of other funding and effort from other sources.

The impact of C.T.R. support is being appreciated and felt by the Veterans Administration, the U.S. Public Health Service and the National Institute of Child Health and Human Development, the Retina Foundation, Massachusetts Eye and Ear Hospital, Massachusetts General Hospital, Harvard School of Public Health, Harvard University Department of Anthropology, Boston University Department of Sociology and Anthropology, Harvard School of Dental Medicine, Tufts University School of Dental Medicine, Boston V.A. Hospital Special Laboratory, Veterans Administration Central Office, U.S.P.H.S. Hospital, Baltimore, and National Institute of Dental Research.

An additional indirect benefit may be mentioned. As a result of these informally coordinated efforts, monitored by the CTR staff, a number of our grantees are serving on advisory committees which appear to be influencing programs underway or contemplated by other agencies.

1003546690

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

It should also be noted that the methods developed by Mr. Enslein are being used to evaluate data related to smoking habits supplied by Dr. C. B. Thomas of the Johns Hopkins University Medical School, which had not been fully evaluated before.

A small portion of the data obtained by Drs. Sanford Chodosh and Maurice Segal is also now being evaluated by these new techniques.

Reference: Progress Report

The committee has received Progress Report No. 2, which covers the period from July 1, 1964 through June 30, 1967.

In this rather long document the part marked "Report 3.1 Accomplishments, F.Y. 1967" describes the new approaches being developed and used. In particular, the section identified as Longevity Study by Charles Rose is relevant to CTR interests.

It is the staff's opinion that the history of this project shows results beyond our expectations. The concept of using small sums to "seed" possible new approaches that may later be incorporated into a larger ongoing investigation is very appealing. The assurances of good judgment underline this group's past activities and projections for the future. Though the application is for a twelve-month period we expect, from performance, that these funds will be used sparingly and judiciously.

The budget understandably cannot be explicitly spelled out in the usual format, but the general target areas are outlined under Item 8 and appended Item 13.

This project had the sympathy of Dr. E. B. Wilson, Chairman of the original subcommittee, as a case in which a small "piggy-back" grant might provide opportunity to correlate smoking habits with a large body of other data collected at the expense of other agencies. His prophetic judgment appears to have been justified.

Vincent F. Lisanti, D.M.D.
Robert C. Hockett, Ph.D.

1003546691

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

EPIDEMIOLOGY, STATISTICS (Methodology)

ORIGINAL

No. 455R1
Activated: 4/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Sommers, Chm.
Dr. Cattell
Dr. Little

Application For Research Grant

Date: August 25, 1967

1. Name of Investigator(s): (Include Title and Degrees)
Co-principal Investigators: Benjamin Bell, M.D. and Charles L. Rose, A.M.: Director and Assistant Director respectively of Normative Aging Study.

2. Institution & Address:
Veterans Administration Outpatient Clinic
17 Court Street, Boston, Massachusetts 02108

3. Short Title of Project:
Supplementary Assistance to the Normative Aging and Longevity Study.

4. Proposed Starting Date:
October 1, 1967

5. Anticipated Duration of this Specific Study:
Twelve months

6. Brief Descripton of Objectives or Specific Aims:
The objectives and specific aims of the Normative Aging Study have already been inserted into the literature under the title: "The Veterans Administration Longitudinal Study of Healthy Aging", and reprints are attached. Briefly, this is a large scale longitudinal aging study sponsored by the Veterans Administration with a budget in excess of $154,000 per year. The supplementary assistance we are requesting is primarily for "seed money" purposes. The Study is still in its beginning formative stages, and is expected to grow and enlarge particularly over the next five years. "Seed money" at this early stage will assist us in making possible this development. In addition to the "seeding" concept through which small grants make possible subsequent budgetary development many times over, the Normative Aging Study as an ongoing program also makes possible the "piggy backing" principle, through which modest funds from funding source A (e.g. The Council for Tobacco Research) are added to the larger funds already appropriated by funding source B (e.g. The Veterans Administration) so that the net result is many times the augmentation factor contributed by A. We are also stretching our research dollar through our policy of cross-linking with other studies. We utilize the sophisticated resources of the Retina Foundation in ophthalmological research at negligible cost to the Study. The Retina Foundation benefits by having our study population made available to them including administrative services of scheduling and record keeping as well as other data which have been collected on our Subjects. The same sort of arrangement has been worked out with the Psychiatric Department of the Mass. General Hospital in the field of Information Processing of the Central Nervous System. Most recently we have linked up with the Longitudinal Studies of the Gerontology Branch of NICHD in Baltimore through the establishment of a Joint Scientific Committee so that these two major studies might coordinate their procedures and produce findings on combined data.

For the rationale and accomplishments of the Normative Aging Study, the reader is again referred to the attached reprint: "The Veterans Administration Longitudinal Study of Healthy Aging". Additional material on the development and accomplishments of the Study are contained in the official reports of the last three years, and copies

7. Give a Brief Statement of your Working Hypothesis:

10035496692

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)
Continuation of 6&7.

of these are also attached. A special aspect of the Normative Aging Study is the problem of developing statistical tools for multivariate analysis, and development of the more involved and virtually unknown area of multivariate treatment of time series (longitudinal) data. The solutions to these problems will be applicable to all complex aging and other biomedical studies whether they take place at one point in time or multiple points in time.

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

Funds requested to carry out the proposed procedures are described in attachement sheet. The emphasis of requested funding is in the exploration of feasibility and development of methodology.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location):

The physical facilities of the Boston VA Outpatient Clinic and those of collaborating investigators in nearby hospitals and universities are available.

10. Additional Requirements:

The continuing relationship of the Council for Tobacco Research with Kurt Enslein, an internationally known specialist in biomedical applications of computers, is indispensable to the development of our data acquisition and computational program. The statistical, programming, and computer resources offered by Mr. Enslein is at the top of the "State of the Art" in computer technology. For example, Mr. Enslein's data link with the computer of the Smithsonian Astrophysical Laboratory in Cambridge provides the best computational services of its kind available anywhere.

11. Biographical sketches of all principal and professional personnel (append)

Biographical sketches of the principal co-investigators are appended.

12. List of publications: (Five most recent as pertinent) (append).

List of five most recent relevant publications is appended., on attachment sheet.

1003546693

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

See attachment sheets

A. Salaries (Personnel by names)	% time	Amount
Professional		
Technical		
	Sub-Total	
B. Consumable Supplies (list by categories)		
	Sub-Total	
C. Other Expenses (itemize)		
	Sub-Total	
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)		
	Total	

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature] 617-223-2272
Director of Project Telephone

Signature [signature]
Business Officer of the Institution
253-2007 Telephone

1003546694

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Normative Aging Study	Veterans Administration	$154,000	Indefinite
Pending	None			

100354669S

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

ATTACHMENT SHEET

Item 8: PROPOSED PROCEDURES

(1) The funding of a part time technician for neurophysiologic studies at the Massachusetts General Hospital which would amount to 10% of the total costs of the Study. The remainder of the costs would be absorbed by available equipment and investigators at the Massachusetts General Hospital. The Study involves evoked potential and EEG investigations which have never been carried out longitudinally. The evoked potential study consists of electro-encephalographic recording of electrical discharge from the brain evoked by a stimulus. The processing of the data involves a special purpose computer which carries out on line calculations so that directions and amplitudes of the stimulus might be varied.

(2) The funding of development of instrummentation to photograph a magnified image of the peripheral retina and choroid. The funding would involve less than 10% of the total cost with the Retina Foundation picking up the remainder. The Retina Foundation is world famous for its development of instrumentation and has available sophisticated biomedical engineers and well equipped laboratories and workshops. The objective of this proposal is to develop means for objective representation and measurement of the peripheral retinal vasculature and choriocapillaries.

(3) The funding of a special method of pulse registration with automated calculation of systolic slope, as a predictor of peripheral vascular occlusion. The problem here is to fund the services of a part time physician. The sophisticated apparatus to carry out this procedure is already available at the Surgical-Vascular Laboratory of the Peter Bent Brigham Hospital.

(4) Funding of services by part time physicians to accomplish unforseen jobs that come up in research development, such as reading EKG's, interpreting medical records to generate coded quantified data, and X-ray reading.

(5) Funding of technical aspects of manuscript preparation such as photographs, drawings, graphs, slides, bibliographic materials, etc. Also, funding costs of obtaining official documents such as birth and death certificates and hospital records.

(6) Funding of travel for professional meetings and local travel for searching hospital or other official records.

Item 12: Publications

(1) B. Bell, C.L. Rose and A. Damon "The VA Longitudinal Study of Healthy Aging." Gerontologist 6:179-184, Dec 1966.

(2) C.L. Rose, R. Nuttall and K. Enslein "Methodologic Problems in Prediction of Longevity" Proc 7th Int. Congress of Gerontology 7:255-258 Vienna, 1966.

10035466696

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Item 12: Publications (Continued)

(3) C.L. Rose and B. Bell "Selection of Geographically Stable Subjects in Longitudinal Studies of Aging" J.Amer. Geriat. Soc. 12:143-151, 1965.

(4) C.L. Rose "Representativeness of Volunteer Subjects in a Longitudinal Study of Aging" Hum.Dev. 8:152-156, 1965.

(5) C.L. Rose "Social Factors in Longevity" Gerontologist 4:27-37, 1964.

Item 13: Budget

In view of the need for flexibility and the principles of supplementary fund use as described in item 8, it would be difficult a priori to specify each item in the budget, so that a general budget is offered instead:

A total of $18,500 is requested broken down as follows:

A.	Personnel	20%
B.	Consumable Supplies	30%
C.	Other expenses (Services, travel, etc.)	50%
D.	Equipment	0%
E.	Overhead	0%

100354697

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Biographical Sketch

BELL, Benjamin R

DATES	PLACE	POSITION OR DEGREE
1924 to 1928	Boston Latin School.	
1932	Harvard College	A.B., cum laude, in Biochemical Sciences
1936	Jefferson Medical College Philadelphia	M.D.
1936 to 1938	Jefferson Medical College Hospital, Philadelphia	House Officer, (Surgical)
1938 to date	Massachusetts Medical Society; Norfolk District Medical Society; American Medical Association.	Member
1939 to 1940	Massachusetts General Hospital, Boston	Graduate Assistant
1940 to 1942	Boston Dispensary, Boston	Graduate Assistant (EENT)
1941	Rhinoplasty course (Foman).	
1942 to 1946	Military Hospitals, Army of United States	Chief of EENT.
1946 to 1947	Boston City Hospital Aural Service	Basic Science Course
1946 to date	Veterans Administration Outpatient Clinic, Boston	Chief, EENT Service
1960 to date	Veterans Administration Outpatient Clinic, Boston	Chief of Staff
1960 to 1962	Clinical Associate in Otolaryngology, Boston University School of Medicine	
1962 to date	Boston University School of Medicine	Asst. Clinical Professor of Otolaryngology
1962 to date	Boston University School of Medicine	Lecturer on Ophthalmology
1963 to date	Veterans Administration Outpatient Clinic, Boston	Chief, Normative Aging Study

1003546698

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS

Pertzoff, V.A.; and Bell, B.: Un Historique Des Premieres Recherches Sur La Composition De La Caseine, Le Lait, 113:161-172 (March) 1932.

Bell, B.: Office Ophthalmology - Normal Fundi, Mass. G. Practice News, IV, 3:6-9 (May) 1956.

Bell, B.: Office Ophthalmology - Abnormal Fundi, Mass. G. Practice News, IV, 4:3-5 (July) 1956.

Bell, B.: Hill, G.E.; and Wyeth, J.M.: Mobilization of Stapes Operation for Deafness due to Clinical Otosclerosis, Medical Research in the Veterans Administration, 2:736-737 (May) 1959.

Bell, B.: and Covell, L.L.: Effect of Systemic Corticosteroid Therapy on Intraocular Pressure, Medical Research in the Veterans Administration, 2:736 (May) 1959.

Bell, B.; Harrison, R.; Trotter, R.R.; and Wolfe, E.: Aqueour Dynamics in 107 Spanish-American War Veterans, presented at National Veterans Administration Research Conference in Cincinnati December 1961; Abstract, Journal of Gerontology, October 1962.

Harrison, R.E., Wolf, E., and Bell, B.: Ocular studies with special reference to glaucoma in a group of 107 elderly males, Am. J. Ophthalmology 57:235-240, 1964.

Rose, C.L., and Bell, B.: Selection of Geographically Stable Subjects in the Longitudinal Study of Aging, J. of the Am. Geriatric Society, February 1965.

PROFESSIONAL COMMUNITY RELATIONS

Invited Lecturer to Clinical Assemblies (1955, 1960 & 1961) of American Academy of General Practice (Mass. Chapter), Mass. Speech and Hearing Society (1960), Rehabilitation Seminar at Boston College (1961), Medical TV (You and Your Health, 1954), and Radio Programs (Ask the Doctor, and Medical Forum of the Air, 1959 to 1961), Task Force on Aging of Mass. Mental Health Planning Project, 1963-1964.

10035469699

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Name: Charles L. Rose

Address: R

Birth: R R

Current Position: Research Associate, Dept. of Sociology, Tufts University, Medford, Massachusetts (Since April 1960)
Research Social Worker, VA Outpatient Clinic, 17 Court St., Boston, Massachusetts (Since November 1960)
Instructor, Northeastern University, University College, Boston, Massachusetts (Since 1962)

Education: A. B., Harvard, 1937 - Psychology
A. M., University of Chicago, 1946, Graduate School of Social Service Administration

Past Professional Experience: Social Worker, Bureau of Public Welfare, Chicago, 1938-1942; Guidance Counselor, Illinois State Training School for Boys, 1942-1945; Social Worker, VA Hospital, West Roxbury, 1946-1949; Supervisor, VA Hospital, Bedford, 1949-1960; Principal Investigator, "Attitudes of Relatives of Mental Patients", Supported by NIMH under Grant M4026, 1960-1962

Societies: National Association of Social Workers
N. E. Society for Research in Psychiatry
Boston Society for Gerontologic Psychiatry

PUBLICATIONS

1. "Corrective Physical Rehabilitation - An Effective Approach" Mil. Surg. 103:125-135, 1948
2. "Effectiveness of the Hospital Conference in Rehabilitation" J. Occup. Ther. & Rehab. 28:245-259, 1949
3. "A Dynamic Group Experience in a Hospital Paraplegic Unit" Mil. Surg. 109:712-720, 1951
4. "A Study of Readmissions to a Mental Hospital", Bedford Research, August 1952
5. "Return of Psychiatric Patients to the Community" Bedford Research, March 1953

10035467700

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Charles L. Rose: (Continued Publications)

6. "Group Research by Student Social Workers", J. Psychiat. Soc. Wk. January 1954

7. "Social Service and Corrective Therapy" J. Assn. Phys. & Ment. Rehab. 9:85-88, 1955

8. "Relatives' Attitudes and Mental Hospitalization", Mental Hyg. April 1958

9. "Attitudes of Relatives of Mental Patients", read at Mass. Conference of Social Work, Boston, Mass., November 5, 1959

10. "Intercorrelation of Medical, Anthropometric and Social Indices in Octogenarians", read at International Congress of Gerontology, Copenhagen, August 1963

11. "Impact of Sons' Mental Illness on Older Parents", read at Gerontological Society Annual Meeting, Boston, Mass., November 1963

12. "Social Factors in Longevity", The Gerontologist 4:27-37, 1964

13. "Social Correlates of Longevity", Ch. 5 in B. Kastenbaum (ed), New Thoughts on Old Age, Springer, N. Y., 1964

14. "Selection of Geographically Stable Subjects in the Longitudinal Study of Aging", J. Am. Geriat. Soc. forthcoming

10035467O1

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#523R1 JONES

1003546702

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 10, 1967

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Bing, Dr. Lynch and Dr. Loosli.

FROM: Robert C. Hockett

SUBJECT: Renewal grant application from Oswald Jones, M.D. - No. 523R1.

We enclose herewith a renewal application with covering letter from Dr. Oswald R. Jones of St. Luke's Hospital, New York, New York.

This represents the second year of the two years for which a grant was approved as a contribution to the overall project at the rate of $10,000 per year. It should be noted that the present request is for support for a period of from three to five years at a much higher level of support (of the order of that requested in the original application). Dr. Jones is now working in collaboration with another group at Ithaca who have obtained similar results from the effect of viruses in dogs.

R. C. H.

10035467O3

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

DR. OSWALD R. JONES
71 EAST SEVENTY-FIRST STREET
NEW YORK 21, N.Y.

August 2, 1967

Dr. Robert C. Hockett
Council for Tobacco Research
633 3rd Avenue
New York City

Dear Doctor Hockett:

I am enclosing my application for research grant from the Council. I hope it is properly filled out. If not let me know. As you may remember, we are starting on the second part of a two year grant of $10,000. per annum. As the study has continually grown in size and scope so have the expenses.

Would it be possible for the Council to consider starting this fall the research grant for which this application has been submitted?

During the past year our study has completed the pulmonary tests on thirty-one children ranging in ages seven to fourteen. Twenty of these were controls, healthy children. The remaining eleven were children who had had a period of pulmonary infection previously, which took the form of bronchitis, bronchiolitis, pneumonia or asthma. The etiological factor in most of these was the virus, type not determined.

The controls showed normal pulmonary tests, including diffusion studies, while the others all showed definite signs of lung damage. The results of this study are now being put together for our preliminary paper, a copy of which will be sent to you. We are most encouraged in our findings and shall continue this study. This coming year, due to the establishment of a diagnostic viral laboratory at St. Luke's Hospital, we will be able to identify the type of virus or other organism which has caused the lung damage.

I am enclosing three copies of a report which show canine distemper virus in the respiratory tract of infected dogs. We think it is an amazing piece of work from our Ithaca study on the effects of canine distemper on the respiratory tract. As distemper in the dog is practically the same as measles in the child, we can safely conclude that the respiratory tract of children probably is injured by measles and similar other viruses. Our original thoughts held that pulmonary emphysema probably was caused by childhood viral infections, such as measles, chicken pox and respiratory syncytial viruses. Our animal and human studies are beginning to show a definite trend in that direction.

1003546704

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

DR. OSWALD R. JONES
71 EAST SEVENTY-FIRST STREET
NEW YORK 21, N.Y.

I do hope that the Council will affirm this request for a research grant which will make it possible to continue our present well oriented studies and to augment them through virological studies.

Sincerely,

Oswald R. Jones, M.D.

10035467O5

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

VIRUS, PREVIOUS INFECTION

NO. 523-R1
Activated: 10/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE
Dr. Jacobson, Chm.
Dr. Bing
Dr. Lynch
Dr. Loosli

Application For Research Grant

Date: August 1, 1967

1. Name of Investigator(s): (include Title and Degrees)

Oswald R. Jones, M.D. - Director of Research Project

2. Institution & Address: St. Luke's Hospital - West 113th Street, New York City

3. Short Title of Project:

Carbon monoxide uptake and diffusion capacity in children. This test together with others will be used to evaluate pulmonary function in children starting at the earliest age of cooperation. This evaluation of pulmonary function will be applied to normal children, those with asthma bronchitis, or chronic pneumonia which conditions were subsequent to infections of unknown, particularly those caused by known viral agents.

4. Proposed Starting Date: October 1, 1967

5. Anticipated Duration of this Specific Study: 3-5 years.

6. Brief Descripton of Objectives or Specific Aims:

Measurements of carbon monoxide (CO) uptake and diffusion capacity (1) have been modified to obviate painful arterial puncture by the use of end tidal PCO at rest (2, 3, 4, 5) and during graded hyperventilation (6). Application of these methods in the dog, and in normal humans and patients with chronic chest diseases have established the relationship between CO uptake and diffusion as graded hyperventilation in normal and diseased adults (7, 10 supporting 8,9)

Normal children will be grouped according to age and size and normal values for CO uptake and diffusion established as controls. Children with asthma bronchitis and post viral pneumonitis will be studied and the results compared.

7. Give a Brief Statement of your Working Hypothesis:

The objectives of the study are: (1) to establish normal values for CO uptake and diffusion capacity at rest and during hyperventilation in children. (2) To study the relationship of asthma, bronchitis and viral pneumonitis on diffusion capacity in children as a background for the development of chronic pulmonary insufficiency.

1003546706

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See attached separate page

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical location)

The cardiopulmonary laboratory of St. Luke's Hospital is a recognized research laboratory in pulmonary diseases with four full time physicians, three blood gas technicians and all equipment and facilities to perform the studies outlined. New CO measurement and recording equipment is requested for specific application to small children and infants.

10. Additional Requirements

The pedicatric department of St. Luke's Hospital is headed by a full time director and operates an active inpatient service of fifty-four beds with 1500 patients per one year, as well as an outpatient department with more than 30,000 visits per one year. A full time pediatric cardiologist will devote part time (1/3) to this project.

10. Additional Requirements

Antibody titre for viruses. Fluorescent antibody search in tissues obtained by biopsy or at post mortem, also cultures of the above tissues for presence of bacteria and viruses.

As mentioned CO uptake and diffusion capacity techniques have been developed in this laboratory which have been validated in adults and which are specifically applicable in children as described.

11. Biographical sketches of all principal and professional personnel(append)

12. List of publications: (Five most recent as pertinent) (append)

10035462702

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Material and Method

Twenty healthy boys, age 9 to 14 years, from the Cathedral School, and 11 patients from the Pediatric Department of St. Luke's Hospital of a similar age group were studied, for their lung function. Studies consisted of extensive spirometric measurements, lung volume evaluation using helium-dilation method, diffusion studies using carbon monoxide method modified in such a way that correction for CO back pressure was introduced and that multiple results on one person were obtained using graded hyperventilation.

History and clinical examination were performed on all healthy boys. All hospital patients were examined and evaluated by a pediatrician. Hospital patients were categorized as 1) normal, 2) those with minimal lung damage, if any, 3) those with mild lung damage, and 4) those with moderate lung damage. Evaluation of the physiologic studies was done independently, without knowledge of the clinical impressions, and the results were then compared.

Observations

Normal boys: Spirometric and volumetric studies have shown increase in lung volumes related to the growth of the person studies, as expected. The ratios of the subdivisions of the lung volumes did not show any significant difference in this particular span of years.

Diffusion studies have shown an unexpected decline in efficiency of the alveolocapillary membrane when expressed per unit of lung volume for more mature boys. The findings indicate that the size of alveoli changes with age, so that actual alveolocapillary membrane reduces per unit of volume. This can be explained by increase of the size of the alveoli. This is, to our knowledge, the first time that this has been shown physiologically, although this was to be expected because the growth of the lungs by proliferation of the alveolar units stops approximately at the age of ten and after that the lung volume increases essentially by expansion of the alveolar space.

Sick persons: It was found that the majority of the sick individuals have shown a distorted ratio of lung subdivisions (residual volume:total lung volume), although a discrimination toward normal persons was impossible because of the wide span of normal values.

Diffusion studies were able to differentiate sick persons from their healthy age-mates. Even mildly sick persons had their diffusion values at the lower limit of normal or below that limit.

In conclusion, it is possible to show that we have reliable tools for measuring lung functions. The means are so sensitive that they can show the physiological changes related to the body growth in this age group. In addition, the results can discriminately show pathologic reduction in functions caused by chronic lung disease in children.

More studies will be needed to establish definite span of normal values for each age and size group, as well as to evaluate damage induced by different noxious agents.

1003546708

Added to our investigative facilities, will be a new diagnostic viral laboratory at St. Luke's were we can identify the causative agent of pulmonary damage in childhood.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Dr. Veljko Krstulovic	100	
Dr. Lucy Swift	33 1/3	
		R
Technical		
Mr. William Rosenberg	100	
Sub-Total		R
B. Consumable Supplies (list by categories)	~~21,242.00~~	
Medical gasses valves tubing bags, ets.		2,000.00
Sub-Total		
C. Other Expenses (itemize)	23,242.00	23,242.00
Sub-Total		
D. Permanent Equipment (itemize)		
CO analyzer		2,800.00
Recorder		2,900.00
		28,942.00
E. Overhead (15% of A+B+C)	3486.30	
Total		32,428.30

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	Same	Same		None	Same	26,728.30
Year 3	Same	Same		None	Same	26,728.30

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [redacted]
Director of Project

Telephone

Signature [redacted]
Business Officer of the Institution

Telephone

10035462709

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Emphysema and Measles	Stony Wold Corporation	$9,000	1 year
Pending				

10035467l0

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

OSWALD R. JONES, M.D.

R R Waterbury, Conn. B.S., 1919 Yale College, M.D. Columbia 1923. St. Luke's Hospital., N.Y. med. 1923-25, Bellevue Hosp., N.Y. chest serv. 1925-26, ass't path. St. Luke's Hosp., 1926-27. Inst. in Med. Col. Univ. 1927-32. Assoc. in Med. 1932-37. Asst. Clin. Prof. of Med. 1937-62. F.A.C.P. Dipl. Int. Med., Ac. Med., Dir. Cardio Respiratory Lab. St. Luke's. Cons. phy. St. Luke's., Cons. Phy. chest dis. Southampton Hosp., Cons. phy. chest service Bellevue Hosp.

References:

1. Jones, O.R.: Measles - A Cause of Emphysema, An Hypothesis Concerning Chronic Pulmonary Emphysema, a Possible Cause and its Prevention, Am. Rev. Resp. Dis. Vol. 87, 4, Apr. 1963

2. Jones, O.R., Platt, W.D., and Amill, L.A. - Miliary Tuberculosis Caused by Intravenous Self-Injection of Tubercle Bacilli, Treated Successfully with Streptomycin Therapy., Am. Rev. of T.B., Vol. 60, No. 4 Oct. 1949

3. Jones, O.R. and Cournand A. - The Shrunken Pulmonary Lobe with Chronic Bronchiectasis., Am. Rev. of T.B. Vol. 28, No. 3 Sept. 1933

4. Miller, J.A., and Jones, O.R. - Primary Carcinoma of the Lung. Am. Rev. of T.B. Vol. 21, No. 1, Jan. 1930

5. Jones, O.R. and Burford, G.E. - Massive Atelectasis following Cyclopropane Anesthesia - Jour. A.M.A. Vol. 110, Apr. 2, 1938.

10035467 11

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

LUCY H. SWIFT M.D.

R R New York City, Barnard College, New York City 1949, A.B., College of Physicians & Surgeons, Columbia University, N.Y.C. M.D. 1953. Mary Imogene Bassett Hospital, Cooperstown, N.Y. 1953 (July-Dec.) St. Luke's Hosp. N.Y.C., 1960-62, clin. fellow pediatric cardiology 1962-1964, Presbyterian Hosp. Babies Hosp. N.Y.C., Res. Assoc. pharm. 1954-1955 Div. Exp. Chemotherapy, Sloan-Kettering Inst. N.Y., Med. Lic. N.Y.S. 1962, Am. Bd. of Ped. Dipl. 1965, Instr. Pediatrics Columbia Univ. 1964-, Asst. Att. Ped.(Cardio) St. Luke's Hosp. 1964-, Ass. Ped. Columbia-Pres. Med. Center, N.Y.C. 1964 - Assoc. Visiting Ped., Harlem Hosp. N.Y. 1965-, Med. Soc. of County of N.Y., N.Y.State Med. Soc. Amer. Med. Assoc.

References

1. Swift, L.H., and Griffiths, S.P.: Cardiac Arrhythmias in the First Year of Life; The Heart Bulletin 14: 113-116, 1965 (Nov. Dec.)

10035467 12

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Veljko Joseph Krstulovic, M. D.

Chronological Listing of All Activities Since Graduation From Professional School

FROM Month	FROM Year	TO Month	TO Year	Type of Activity, including Name and Address of Employer, Beginning with Date of Graduation from Professional School
	1936			Baccalaureate from Second State's Boys Gymnasium, Zagreb.
Oct.	1941			Graduation from Medical School of the University of Zagreb.
Nov.	1941	May	1942	Internship in Pediatrics, Dermatology & Gynecology at The University of Zagreb.
May	1942	Feb.	1943	Continuation of Internship in the Chief Army Hospital, Zagreb.
				Postdoctoral Training
Aug.	1947	Oct.	1947	Course in Aviation Medicine, Zemun, Yugoslavia
July	1950	Nov.	1951	While in the army, attended daily medical conferences, ward rounds, cardiac conferences and EKG reading in Medical Department of University Hospital in Zagreb. Chief, Dr. Ivan Botteri, Professor of Clinical Medicine.
Dec.	1951	Dec.	1954	Residency in Internal Medicine at University Hospital. Chief, Dr. Ivan Botteri, Professor of Clinical Medicine, succeeded by Dr. Arpad Hahn, Professor of Clinical Medicine.
Jan.	1955	Dec.	1956	Residency in Cardiology at University Hospital. Dr. Radovan Ivancic, Associate Professor in Medicine.
July	1957			Board of Internal Medicine. Croatia-Yugoslavia.
				Professional Appointments
Feb.	1943			Medical Officer in Yugoslav Army.
April	1945	July	1947	Medical Officer in Air Force battalion (equivalent to flight surgeon in U. S. Air Force).
July	1947	Nov.	1951	Chief of Clinical Medicine in Air Force Division.
Dec.	1951	Jan.	1957	Instructor in Medicine at Medical School, University of Zagreb. Chief, Dr. Ivan Botteri and Dr. Arpad Hahn, Professor in Clinical Medicine.

1003546713

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Veljko Joseph Krstulovic, M. D.

FROM		TO		Type of Activity, including Name and Address of Employer, Beginning with Date of Graduation from Professional School
Month	Year	Month	Year	
Feb.	1957	June	1960	Chief of Cardiopulmonary Section of Internal Medicine Polyclinic at Medical School in Zagreb. Chief, Dr. Vinko Vuletic, Professor of Clinical Medicine.
July	1957	June	1960	Associate Supervisor in Charge of Physical Diagnosis Course at the Medical School in Zagreb. Chief, Dr. Vinko Vuletic, Professor of Clinical Medicine.
July	1960	June	1961	Research Fellow at Cardiovascular Lab at Cornell Medical School. Chief, Dr. Irving S. Wright, Professor of Clinical Medicine, Cornell Medical School.
July	1961	Dec.	1964	Research Fellow in Cardiopulmonary Laboratory at St. Luke's Hospital Center, New York City. Chief, Dr. A. L. Loomis Bell, Jr.
Jan.	1965	present		Research Associate in Cardiopulmonary Laboratory at St. Luke's Hospital Center.
May	1966	present		Consultant for Heart and Lung Diseases, Harlem Hospital.

10035467 14

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Veljko Joseph Krstulovic, M.D.

List of Publications in Yugoslavia

1) Krstulovic, V.: Dyspnea as a medical emergency. Radovi Medicinskog Fakulteta u Zagrebu. 1: 78, 1955.

2) Krstulovic, V., Ivancic, R.: Hemodynamics of mitral valvular disease. Lijecnicki Vijesnik. 79: 365, 1957.

3) Krstulovic, V.: Cardiopulmonary functions in normals and in subjects with chronic pulmonary diseases. Tuberculosis. 10: 132, 1958.

4) Oberhofer, T., Pitamic, T., Gvozdanovic, V., Krstulovic, V.: Dextrocardia combined with cardiovascular malformations. Pediatria Iugoslavica. 2: 297, 1960.

5) Golinovic, N., Bunarovic, N., Krstulovic, V.: Candida albicans mycelium in the left atrium imitating clinical finding of intermittent mitral stenosis in a case of pulmonary moniliasis. Medicinski glas. 1961.

6) Ivancic, R., Krstulovic, V.: Electrocardiography. Skolska knjiga, Zagreb. 1960.

7) Krstulovic, V.: Venous pressure and circulation time as a differential diagnostic mean in cardiopulmonary disturbances. In press.

8) Krstulovic, V., Gvozdanovic, V., Hauptman, E.: Splenic pulp pressure correlated with splenoportal angiography in patients with portal hypertension and in patients with splenic artery thrombosis. In press.

10035467215

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Veljko Joseph Krstulovic, M. D.

List of Publications in United States of America

1) Shimomura, Seiichi, Pierson, Richard N., Jr., Krstulovic, Veljko, and Bell, A. L. L., Jr.: Primary and secondary pulmonary vasopressor responses to acetylcholine demonstrated by the wedged catheter perfusion technique. (Abstract), Bull. N. Y. Acad. Med., 38: 839, 1962.

2) Bell, A. L. L., Jr., Kightlinger, Benjamin, Shimomura, Seiichi, and Krstulovic, Veljko: Postvalvular pulmonary artery stenosis. Hemodynamic and radiologic definition. Circ. 26: 685, 1962. (Abstract).

3) Shimomura, Seiichi, Krstulovic, Veljko, Pierson, Richard N., Jr., and Bell, A. L. L., Jr.: Separation of the primary vasoconstrictor effect of acetylcholine in the pulmonary vascular bed from systemically induced response utilizing the wedged perfused segment in the intact dog. Circ. 26: 785, 1962. (Abstract).

4) Kightlinger, Benjamin N., Shimomura, Seiichi, Krstulovic, Veljko, Kittredge, Richard D., and Bell, A. L. Loomis, Jr.: Postvalvular pulmonary artery stenosis. Hemodynamic and radiologic definition (Abstract), Bull. N. Y. Acad. Med. 39: 58, 1963.

5) Haynes, W. F., Jr., Krstulovic, V. J., Bell, A. L. L. Jr.: Smoking habit and incidence of respiratory tract infection in group of adolescent males. Am. Rev. Resp. Dis. 93: 730, 1966.

6) Krstulovic, V. J., Haynes, W. F., jr., and Bell, A. L. L. Jr.: Spirometry in adolescent males. I. Normal values. Submitted for publication.

7) Krstulovic, V. J., Haynes, W. F., Jr., and Bell, A. L. L. Jr.: Spirometry in adolescent males. II. Comparison of spirometric data. Submitted for publication.

8) Krstulovic, Veljko: Graded hyperventilation for evaluation of dynamics of diffusion. In preparation.

9) Krstulovic, Veljko J., Felton, Charles, and Bell, A. L. L. Jr.: Effect of respiratory rate on lung diffusion using graded hyperventilation. In preparation.

10) Krstulovic, Veljko J., Jones, Oswald R., Bell, A. L. L. Jr.: Age related changes in the efficiency of the alveolar capillary membrane in children obtained by hyperventilation diffusion study. In preparation.

10035467 16

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#558MR1-FRIEDELL

1003546717

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 3, 1967

MEMORANDUM

TO: The committee comprising Dr. Reimann, Chm., Dr. Jacobson and Dr. Lynch.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Gilbert H. Friedell, M.D. - No. 558-M-R1.

We enclose herewith a renewal application from Dr. Gilbert H. Friedell of the New England Deaconess Hospital, Boston, Massachusetts. This application is accompanied by a progress report.

The present request is for the second year of the program, for which prior consideration in the allocation of funds was promised. It should be noted that the U.S. Public Health service has provided funds for the continuation of the study and Dr. Friedell does not contemplate asking the Council for additional support.

Robert C. Hockett

1003546718

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CLINICAL - PATHOLOGIC

NO. 558-M-R1
Activated: 11/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Reimann, Chm.
Dr. Jacobson
Dr. Lynch

Application For Renewal of Research Grant

First ☒ Second ☐

Date: July 25, 1967

1. Name of Investigator(s): (include title and degrees)

Gilbert H. Friedell, M.D., Senior Research Associate

2. Institution & Address:
New England Deaconess Hospital
Cancer Research Institute
185 Pilgrim Road
Boston, Massachusetts 02215

3. Short Title of Project:

The Pathogenesis of Human Bladder Cancer

4. Proposed Renewal Starting Date: (Anniversary or other)
November 1, 1967

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

In this project we propose to study the pathogenesis of cancer of the urinary bladder in human patients. We propose to investigate the role, if any, of cigarette smoking in this disease, but only as part of a broader investigation of etiologic factors. For this reason our Detailed Plan of Procedure is divided into the following sections:

Introduction

Specific Aims

Methods of Procedure

Previous Work in this Field

Selected Personal Publications

6. Give a Brief Statement of your Working Hypothesis if altered or modified:
Not modified, see #5 above.

100354219

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

None

8. Additional Requirements:

None

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

No changes

10. Publications or Papers in Press resulting from the Project or closely related work

None at this time.

10035467720

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year)

A. Salaries (Personnel by names or category)	% time	Amount
Professional		
Pathologist, part-time		$ 6,000
Technical		
Interviewer		5,000
Research Assistant		6,000
Laboratory Technician		4,500
	Sub-Total	21,500
B. Consumable Supplies (list by categories)		
Laboratory & office supplies		1,000
	Sub-Total	1,000
C. Other Expenses (itemize)		
Shipment of urine samples-local		500
Shipment of urine samples-Madison		1,000
Travel expenses for interviewer		1,000
Epidemiological and statistical consultations		2,000
	Sub-Total	4,500
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+ B +C)		4,050
	Total	31,050

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature _____ Telephone
Director of Project

Signature _____ Telephone
Business Officer of the Institution

10035497 21

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	The Pathogenesis of Human Bladder Cancer	National Institutes of Health National Cancer Institute	$149,188 (D.C.)	4/1/67- 3/31/71
Pending				

10035462222

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CTR GRANT NO. 558

PROGRESS REPORT NO. 1

G. H. FRIEDELL, M.D.
Cancer Research Institute
New England Deaconess Hospital
194 Pilgrim Road
Boston, Massachusetts 02215

November 1, 1966 - June 30, 1967

THE PATHOGENESIS OF HUMAN BLADDER CANCER

The specific aims of this project were to study the relationship between:

1. Detailed smoking, occupational, residential and family histories;
2. Levels of urinary excretion of urinary metabolites; and
3. The pathology and clinical course of patients with bladder cancer.

I will briefly discuss our progress with regard to each of these specific aims.

Interview Studies

We have interviewed 141 patients with bladder cancer, 106 males and 35 females. A majority of interviews were patients originally treated at either the New England Deaconess or New England Baptist Hospitals, but recently we have begun to interview patients treated in the Boston Veteran's Administration Hospital as well. We are pleased at gaining access to this latter group of patients for we feel it represents a different population than we had previously been studying. For example, in 4 of the first 16 cases seen at the V. A. Hospital a history of employment in the rubber industry was found.

1003546723

We have interviewed 176 control subjects, 90 male and 86 female. The selection of an appropriate control population for such an interview study has been somewhat difficult, however, and we have enlisted the assistance of epidemiologists at the Harvard School of Public Health in making this selection. They have been of great help to us, but in addition, and perhaps of greater significance, I believe members of that department have become sufficiently interested in exploring the epidemiology of

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

bladder cancer to collaborate in a more extensive interview study. This study would cover the greater Boston area and should more accurately reflect the possible influence of environmental factors in the pathogenesis of this disease.

Thus far 34% of the females and 75% of the male bladder cancer patients who have been interviewed have smoked. This is approximately the same ratio as in our control population, but at present we are attaching no significance to these figures. No significant trend is yet apparent in our data concerning occupational or residential history but 48% of the female and 48% of the male population with bladder cancer have a family history of cancer. Again I am reluctant to comment about the significance of these numbers until we have reviewed our data with our statistical consultants.

By the end of the period covered in this report we had obtained the services of a second trained interviewer and with the additional funds furnished by The Council For Tobacco Research-USA we had obtained the part-time services of Dr. Carl Cornil, a trained urologist.

Tryptophan Metabolites

With regard to tryptophan metabolite excretion we have carried out 24, 48 or 96 hour studies on 63 patients and 32 controls. Not all of the urine samples have been assayed but thus far it would appear that approximately one third of the patients with bladder tumors have abnormal excretion of tryptophan metabolites. If one excludes patients with papillomas from the group of tumor cases, approximately one half of the remaining patients have abnormal values. This latter figure is similar to the one originally published by Price and his co-workers for the 41 Madison patients with bladder tumors.

1003546724

In our material it would appear that the histological grade and extent of the tumors are related to abnormal urinary metabolite concentrations. A review of the original pathological material from Dr. Price's group of 41 cases suggests that in

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

this group also the extent of the tumor was related to the presence or absence of abnormal urinary metabolite values. This review was conducted by Dr. Safouh Atassi and me in Madison within the past few months.

Pathology and Clinical Course

Dr. Spencer Burney and I, in Boston, have almost completed our review of the pathology from all of the bladder tumor patients whom we have thus far interviewed. In addition we are almost through with a review of 25 cases in which giant histologic sections were prepared. These latter studies should be suitable for publication within the next few months.

A great deal of time and energy has been spent following up the histories of all patients treated for bladder cancer at the New England Deaconess and New England Baptist Hospitals. From this review the fact has emerged that the majority of patients surviving their primary therapy will be found in the first 5 years after treatment. We will shortly have fairly complete information about 5, 10 and 15 year survival in bladder cancer patients treated at these two institutions. Such information has heretofore not been available.

Financial Support

Finally, it should be noted that we have been awarded a research grant from the U. S. Public Health Service to continue our bladder cancer studies for a 4 year period. This grant will supplement funds from The Council For Tobacco Research-USA for the first 2 years and then will furnish entire support for the last 2 years of our proposed research project. The grant year for the Public Health Service award began April 1, 1967. The sums awarded for each of the 4 grant years are $31,307.00, $46,935.00, $57,185.00, and $13,761.00.

At this time we hope that The Council For Tobacco Research-USA will see fit to renew its award to us for a second year beginning November 1, 1967. We do not anticipate asking The Council for additional support for this project beyond the second grant year.

1003546725

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#561R1 BRINKMAN

1003546726

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. August 1, 1967

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Sommers and Dr. Reimann.

FROM: Robert C. Hockett

SUBJECT: A renewal application from Geoffrey L. Brinkman, M.D. - No. 561R1.

We enclose herewith a first renewal application from Geoffrey L. Brinkman, M.D. of Wayne State University School of Medicine, Detroit, Michigan.

Dr. Brinkman's observation of intracellular structures in the human bronchial mucosa are very exciting and an effort which warrants continued support. Also the opportunity to obtain bronchial biopsies from children undergoing lung resection might provide needed information of normal bronchial structures and their variations.

This application, with a reduced budget, includes a progress report and covers the second of a three-year program for which prior consideration in the allocation of funds was promised.

Robert C. Hockett
John H. Kreisher

10035467727

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY- CARCINOGENESIS & TISSUE CULTURE

NO. 561R1
Activated: 11/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Bing, Chm.
Dr. Sommers
Dr. Reimann

Application For Renewal of Research Grant

First [X] Second [ ]

Date: July 6, 1967

1. Name of Investigator(s): (include title and degrees)
Geoffrey L. Brinkman, M.D., Associate Professor of Medicine

2. Institution & Address:
Wayne State University School of Medicine
1400 Chrysler Freeway
Detroit, Michigan 48207

3. Short Title of Project: The Effect of Cigarette Smoking on the Ultramicroscopic Structure of the Bronchial Mucosa

4. Proposed Renewal Starting Date: (Anniversary or other) December 1, 1967

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

Recently in the bronchial mucosa from an adult, non-smoking patient, some structures have been seen which have the typical morphological appearance of tumor virus (Figures 1 & 2). These virus-like bodies were seen in several cells from this particular biopsy. Whether they are oncogenic or in any way related to bronchogenic neoplasms cannot be decided at this time. However, further study is indicated to see whether these virus-like bodies can be identified in other individuals, both in the bronchial mucosa and in lung tumors. For this reason, I plan to broaden the scope of the study to include study of the ultramicroscopic structure of lung cancers. If these virus-like bodies can be recognized in lung tumors, then further study will be justified to determine whether they have oncogenic properties.

Representative photomicrographs of some recent biopsies are attached (Figures 3-10).

Up until now it has been impossible to obtain bronchial biopsies in normal individuals and we have had to rely on patients who are undergoing lung resection and in whom there was no clinical or pathological evidence of bronchial mucosal abnormality. I have now been able to make arrangements with Children's Hospital of Michigan to obtain bronchial biopsies from children undergoing lung resection for removal of congenital pulmonary abnormalities, and as a result we will be able to obtain bronchial tissue which probably can be regarded as normal.

6. Give a Brief Statement of your Working Hypothesis if altered or modified:

10035546728

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

No basic change in experimental design or procedure is planned, but we have experimented during the year with different strength fixatives and different embedding material (Vestophal and Maraglass). Experience with comparison of these variations of technique are proceeding and ultimately will, I am sure, enable us to determine what is the best procedure for processing human bronchial mucosa.

8. Additional Requirements:

10035467 29

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

My original technician is now working on a part-time basis because of domestic problems, but as well, I have another part-time technician who is an undergraduate student in Biology at Wayne State University. Between the two technicians I have the equivalent of a full-time technician.

10. Publications or Papers in Press resulting from the Project or closely related work

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year)

	% time	Amount
A. Salaries (Personnel by names or category)		
Professional		
Geoffrey L. Brinkman	33-1/3	Nil
Technical		
Mary Ann Perczak	100	7,000.
Social Security		290.
	Sub-Total	7,290.
B. Consumable Supplies (list by categories)		
Photographic		750.
Chemicals, embedding materials		500.
Glassware		100.
	Sub-Total	1,350.
C. Other Expenses (itemize)		
Service Contract		1,400.
	Sub-Total	1,400.
D. Permanent Equipment (itemize)		
Viewing binoculars		563.
E. Overhead (15% of A+ B +C)		1,506.
	Total	12,109.

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature G. L. Brinkman.
Director of Project
Telephone

Signature Clifford M. [illegible]
Business Officer of the Institution
Telephone
833 5733

1003546730

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Gift to purchase electron microscope and to equip darkroom.	Rand Foundation	$39,000.	
Pending	Morphology of the Ageing Lung	Detroit TB & Health Society	$20,795.	1 yr.
	Effect of Cigarette Smoking on Ultramicroscopic Structure of the Human Bronchial Mucosa	American Cancer Society	$11,847.	1 yr.

1003546731

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LEGEND

C = cilia

P = pellicular structures

L = lumen

W = "whiskers"

Dg = degenerating cell

Cm = cell membrane

Mv = microvilli

Ic = intercellular spaces

V = vacuoles

M = mitochondria

Cs = cisternae

Tf = tonofibrils

N = nucleus

D = desmosomes

R = ribrosomes

Sc = scrolls

G = granule

100354732

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546733

FIGURE 1. A cell in the bronchial mucosa showing large cisternae which probably represent Golgi organ. In the surrounding cytoplasm are numerous vacuoles, some of which contain virus-like bodies consisting of a central core with a surrounding two or three-layered concentric wall. These are better seen in the next figure.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FIGURE 3. This photomicrograph was obtained from a man who was a heavy smoker and shows the luminal surface of a ciliated cell with several cilia which appear normal and a great deal of pellicular material between the cilia. This pellicular material is a branching form of particularly large microvilli and greatly increases the specific surface area of the cells. On the right hand side there is a dark cell which is an exhausted goblet cell. The microvilli on this cell are covered with "whiskers", which are seen in greater detail in Figure 4.

10035462234

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

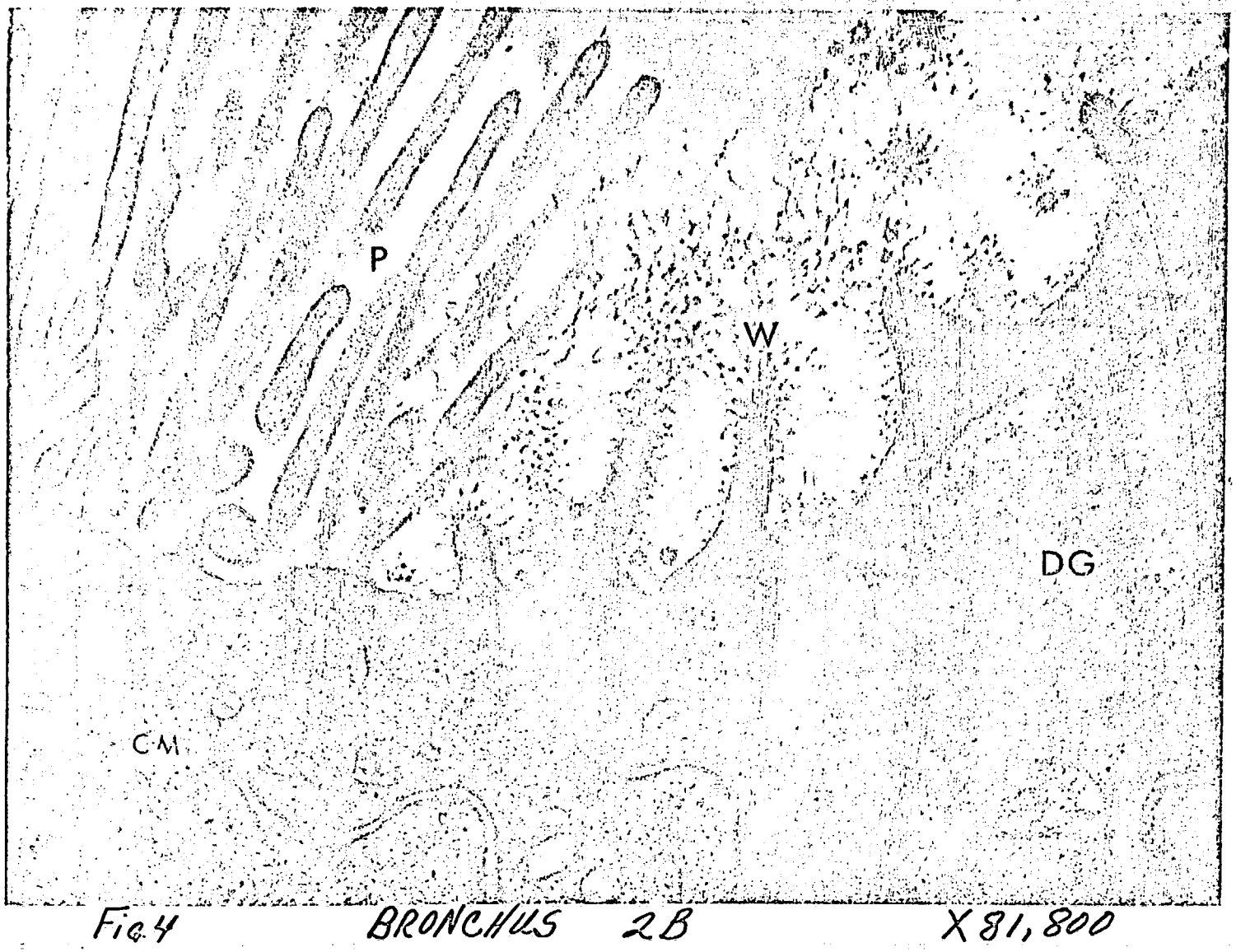


FIGURE 4. Shows the microvilli with "whisker" formation. This has been noted frequently on cells which appear to be exhausted and about to be shed into the bronchial lumen. On the left, the pellicular material is seen in greater detail.

1003549235

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

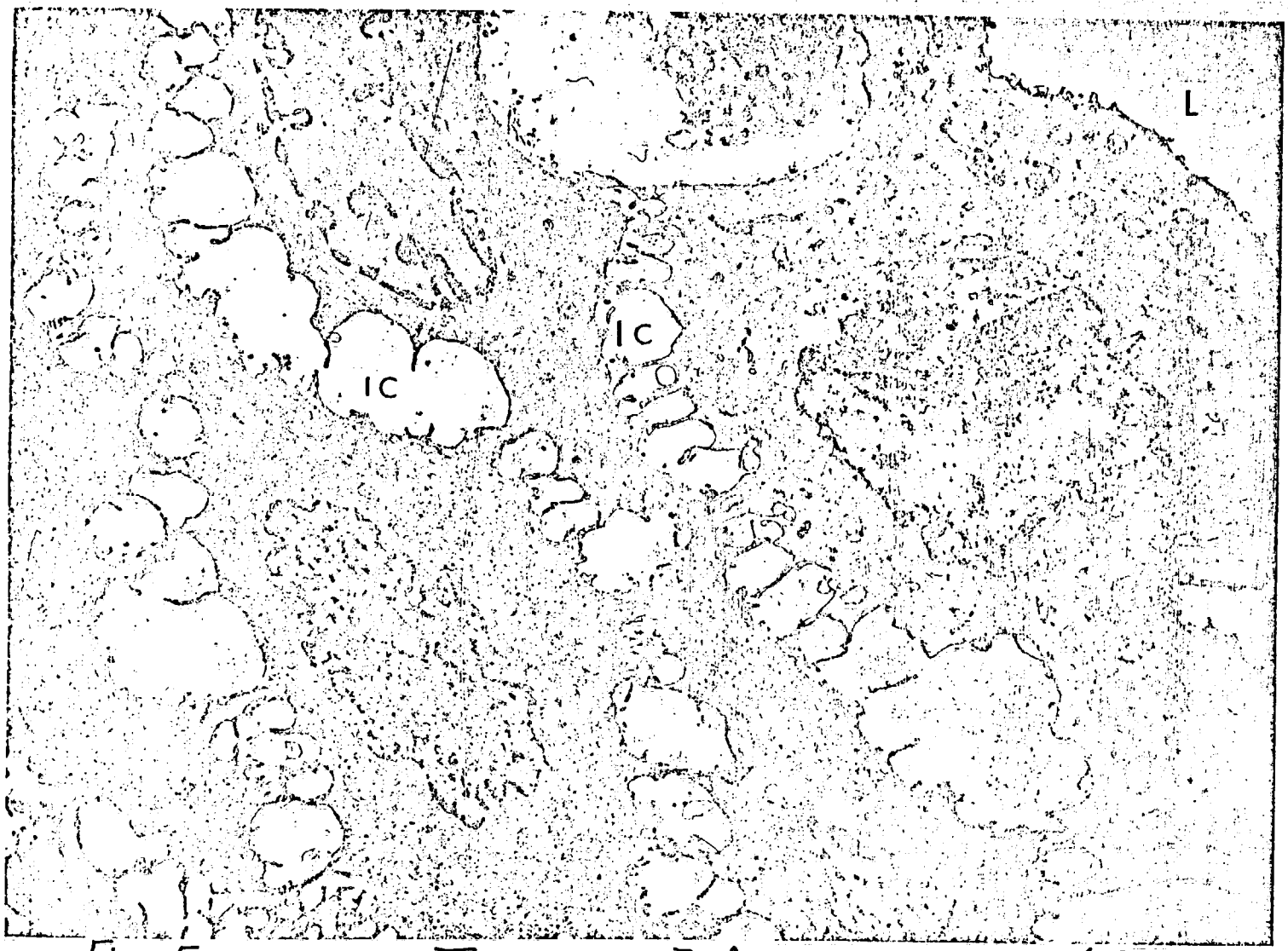


FIGURE 5. This is a cross-section of the tracheal mucosa and shows cells in the process of being shed into the tracheal lumen. The cell nearest the lumen on the right has a light cytoplasm and very little intracellular structure. The intercellular processes which are normally interlocking are well seen.

1003546736

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003543737

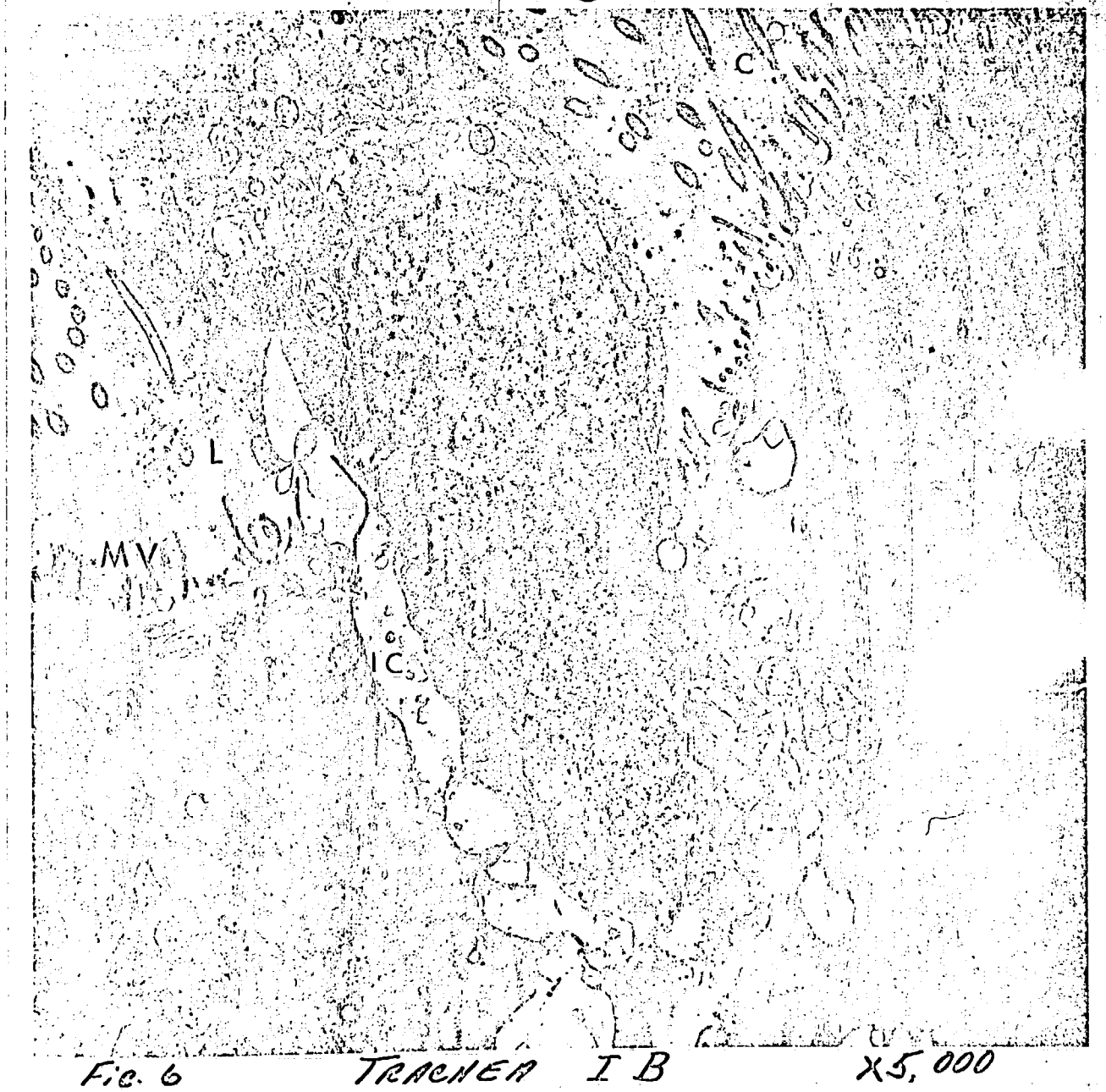
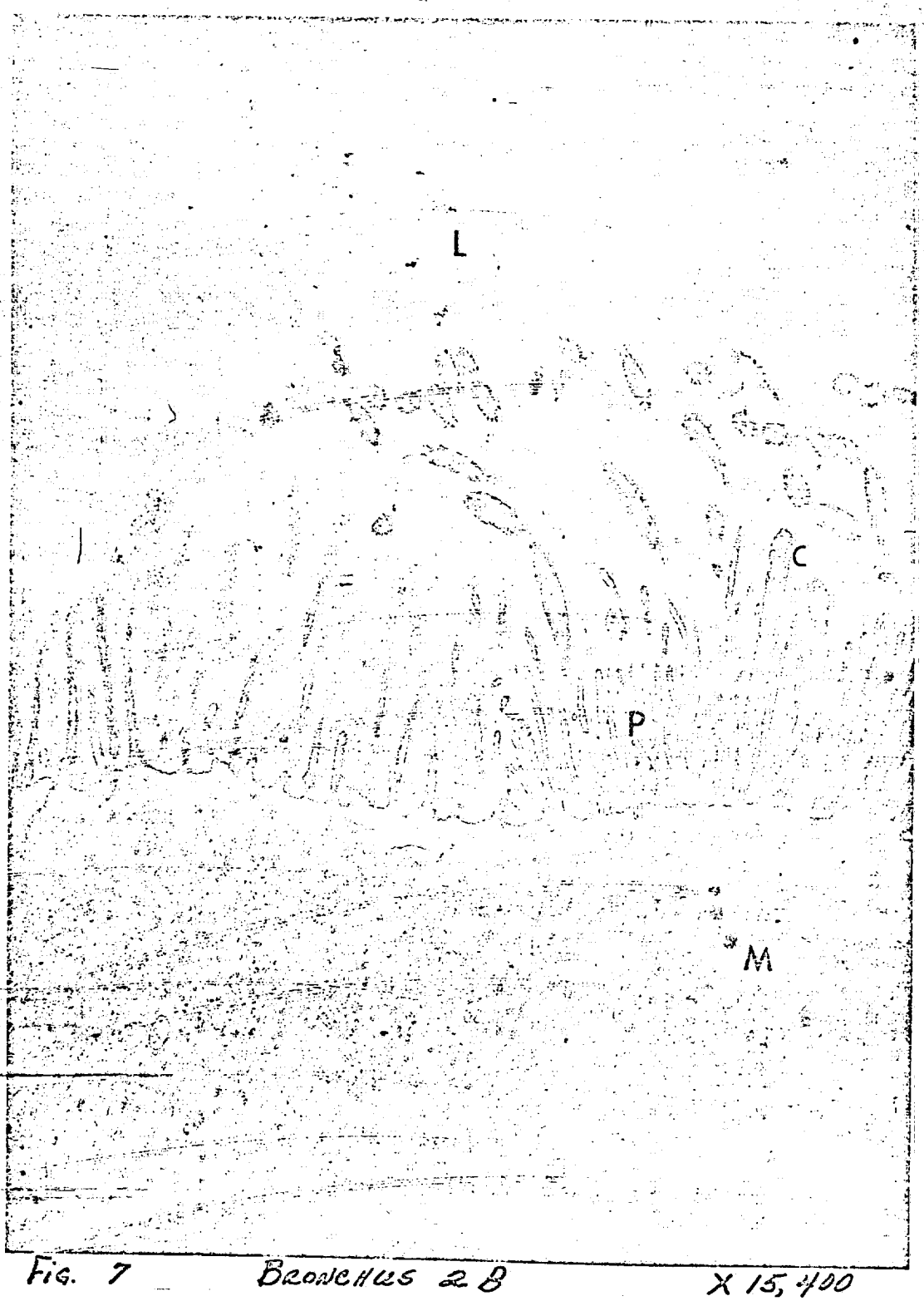


FIGURE 6. This was also obtained from the tracheal mucosa, but is a longitudinal section which shows a cell in the process of being expelled into the lumen.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000



100354738

FIGURE 7. Shows two cells adjacent to the bronchial lumen. The one on the right is a normal ciliated cell with well-formed cilia and pellicular material containing large numbers of mitochondria which extend up close to the basal body of the cilia. The cell on the left has a nude appearance due to the loss of the luminal structures. The mitochondria in this cell are much sparser, giving the cell an empty appearance. This cell is probably in a state of exhaustion and about to be shed into the lumen.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FIGURE 8. Shows tracheal mucosal cells which were obtained adjacent to a squamous cell carcinoma of the trachea. This figure shows well-developed desmosomes, while in the cells themselves there are numerous tonofibrils which may indicate that this cell is reverting to a more primitive state.

1003546739

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

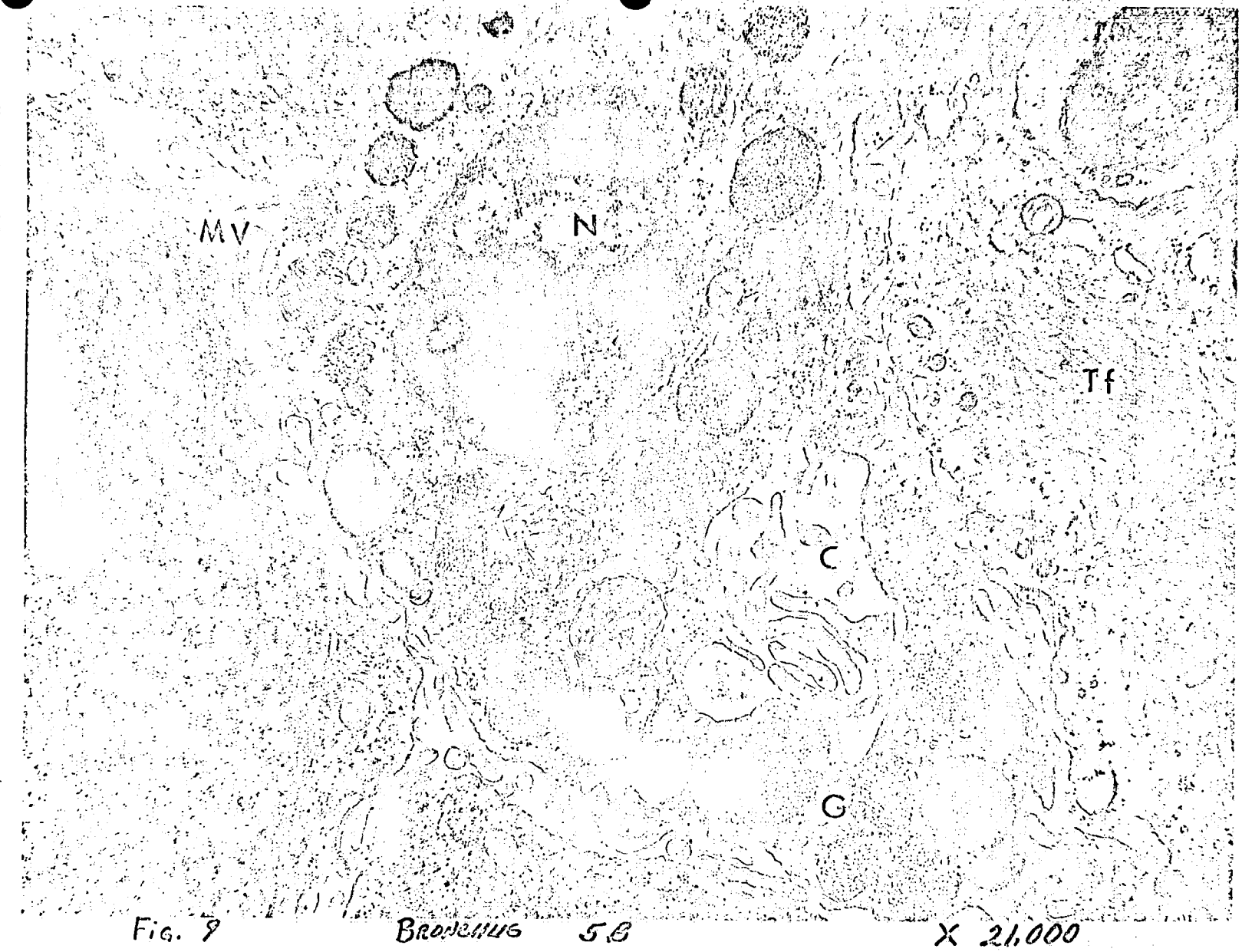


FIGURE 9. The next four photomicrographs were obtained from grossly normal bronchial mucosa in a patient with squamous cell carcinoma. This figure shows a large cell containing numerous vacuoles with varying types of substructure within. As well, large cisternae are noted. The cell on the right of this figure shows well-developed tonofibrils.

10035740

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FIGURE 10. Shows the granules seen in the previous figure in greater detail. Within these granules two and three-layered membranes are forming, some of which are concentric and begin to have the appearance of the virus-like bodies seen in Figures 1 and 2.

1003543741

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FIGURE 11. Shows these granules at greater magnification with these unusual scroll-like substructures forming within the granules.

1003546742

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FIGURE 12. Further demonstration of these scroll-like substructures, some of which bear close resemblance to the virus-like bodies. The possibility that this cell is the site of either multiplication or destruction of viruses must be entertained.

1003546743

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#566R1 · LYNCH

1003546744

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 2, 1967

MEMORANDUM

TO: The committee comprising Dr. Reimann, Chm., Dr. Jacobson, Dr. Loosli and Dr. Sommers.

FROM: Robert C. Hockett

SUBJECT: A renewal application from Kenneth M. Lynch, M.D., and Forde A. McIver, M.D. - No. 566-R1.

We enclose herewith a grant renewal request from Dr. Kenneth M. Lynch and Forde A. McIver of the Medical College of South Carolina, Charleston, South Carolina.

This represents support for the final year of an original two-year request for which prior consideration was promised in the allocation of available funds.

Also enclosed is a five-month progress report through July 15, 1967 and a copy of a M.S. Thesis by Martha R. McGee, entitled "Asbestos as a Possible Co-Carcinogen" representing effort on the current and previous project.

Robert C. Hockett
John H. Kreisher

10035467 45

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

No. 566-R1
Activated: 10/1/66
Cf. #276
Activated: 6/1/60
Renewed annually as of June 1, 1961-1965 inclusive.
Supplement: 6/1/66

COMMITTEE
Dr. Reimann, Chm.
Dr. Jacobson
Dr. Loosli
Dr. Sommers

Application For Renewal of Research Grant

First ☒ Second ☐

Date: 26 July 1967

1. Name of Investigator(s): (include title and degrees)
   Kenneth M. Lynch, M.D., Emeritus Professor of Pathology
   Forde A. McIver, M.D., Associate Professor of Pathology
2. Institution & Address:
   Medical College of South Carolina
   80 Barre Street
   Charleston, South Carolina 29401
3. Short Title of Project:
   Environmental Factors and Pulmonary Carcinogenicity
   I. Asbestos Dust
   II. Asbestos and Co-carcinogens, Viral and Chemical
4. Proposed Renewal Starting Date: (Anniversary or other)
   1 October 1967
5. Discuss any Important Changes or Additions to Objectives or Specific Aims:
   No significant changes or additions to objectives or specific aims.
   (See Progress Report)

1003546746

6. Give a Brief Statement of your Working Hypothesis if altered or modified:
   No alteration in working hypothesis.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

No major changes in experimental design, but this feature is restated and brought up to date in the Progress Report.

8. Additional Requirements:

Except for the continued advice and assistance of the Staff of the Oak Ridge National Laboratories, no additional requirements are anticipated. The desire to report portions of this work at the Second International Conference on the Biological Effects of Asbestos in Dresden, Germany in April 1968 is reflected in the budget request.

10035497A7

9. Changes in Personnel with Biographical Sketches of new Personnel (append) No changes in professional personnel. Mr. Grimsley, who served as Project Assistant last year, has entered graduate school and Mr. Cummings has taken over both positions. Heyward Wesley has replaced Ben Simmons as Animal Caretaker. Paul Gillette and James Powers, medical students, have been employed to take care of animals and supervise the project on week-ends and holidays.

10. Publications or Papers in Press resulting from the Project or closely related work

Manuscript on exposure of dogs to asbestos dust in preparation for presentation at Second International Conference on the Biological Effects of Asbestos. Manuscript (thesis) on Asbestos as a Possible Co-carcinogen completed by Martha R. McGee (see attached copy).

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year)

A. Salaries (Personnel by names or category) Professional	% time	Amount
Kenneth M. Lynch, M.D.		0.00
Forde A. McIver, M.D.		0.00
Technical		
(See attached page 1)		14,340.00
	Sub-Total	
B. Consumable Supplies (list by categories)		
(See attached page 1)		12,587.00
	Sub-Total	
C. Other Expenses (itemize)		
(See attached page 2)		1,395.00
	Sub-Total	
D. Permanent Equipment (itemize)		
None		0.00
E. Overhead (15% of A+ B +C)		4,248.00
	Total	32,570.00

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Forde A. McIver
Director of Project
Forde A. McIver, M.D. 723-9411
Telephone

Signature John E. Wise
Business Officer of the Institution
John E. Wise, 723-9411
Telephone
Vice President for Business Affairs

10035748

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year)

A. Salaries - Technical

	% of time	Amount
Mrs. Patricia Dumas	100	4,800.00
Michael Cummings	100	3,600.00
Heyward Wesley	100	3,300.00
Ben Martin	2	240.00
Paul Gillette*	10	1,200.00
James Powers*	10	1,200.00
		$14,340.00

*Paid at high rate per hour for week-end and holiday supervision and care of animals.

B. Consumable Supplies (list by categories)*

Animal Care Committee maintenance charge for mice without caretaker @ 0.01 per animal/day	7,020.00
Animal Care Committee maintenance charge for 20 dogs without caretaker @ 0.15 per animal/day	1,026.00
Animal Care Committee maintenance charge for 70 guinea pigs with animal caretaker @ $0.08 per animal/day	2,016.00
Laboratory supplies for light and electron microscopy	1,350.00
Photographic supplies	375.00
Asbestos and dusting supplies	450.00
Animal care supplies - gloves, shoe covers, coveralls, disinfectants, etc.	350.00
	$12,587.00

1003546749

*Please note that new Medical College policy has resulted in a sharp increase in the cost of animal care. Nevertheless, these charges are 25% to 40% below those of neighboring medical colleges.

C. Other Expenses (itemize)

Travel to report on previous studies at the Second International Conference on the Biological Effects of Asbestos and for advice and consultations at Oak Ridge National Laboratories	875.00

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

C.	Other expenses (cont'd)	
	Services by Surgery Department for broncho-scopic studies	120.00
	Maintenance of dusting equipment	400.00
	Sub-total	$1,395.00
D.	Permanent equipment (itemize)	
	None Sub-total	0.00
e.	Overhead (15% of A+B+C)	4,248.00
	Total	$32,570.00

10035462S0

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	No other funded research at this time.			
Pending	No other research applications pending.			

10035467S1

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546752

#567R1 SEVERI

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

July 25, 1967

TO: The committee comprising Dr. Little, Chm., Dr. Sommers, Dr. Reimann and Dr. Loosli.

FROM: Robert C. Hockett

SUBJECT: Renewal application from Lucio Severi, M.D. - No. 567-R1.

We enclose herewith a renewal application from Professor Lucio Severi, Professor in the Division of Cancer Research, University of Perugia, Perugia, Italy. It represents the first renewal of an original three-year request for which prior consideration was promised in the allocation of available funds. The amount requested is $1,000 less than was granted for the current year.

This study seems to be going forward according to plan. Under item #10 of the application, three papers are listed. The first Progress Report (October 1, 1966 - April 1, 1967) was distributed to the Scientific Advisory Board on June 23, 1967.

R. C. H.

10035467753

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

NO. 567RL
Activated: 10/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Little, Chm.
Dr. Sommers
Dr. Reimann
Dr. Loosli

Application For Renewal of Research Grant

First ☒ Second ☐

Date: 19th July, 1967

1. Name of Investigator(s): (include title and degrees) Prof. Lucio Severi, M.D. Principal Investigator; Cesare Biancifiori, M.D. Co-Principal Investigator; Rodolfo Ribacchi, M.D. Assistant; Emilio Bucciarelli, M.D. Assistant; Gaetano Giraldo, M.D. Assistant

2. Institution & Address:
Division of Cancer Research, University of Perugia
P.O. Box 327 PERUGIA ITALY

3. Short Title of Project:
An Approach to the Study of Internal Factors in Lung Carcinogenesis. Influence of Hormones.

4. Proposed Renewal Starting Date: (Anniversary or other) October 1st, 1967

5. Discuss any Important Changes or Additions to Objectives or Specific Aims: There have been no basic changes made to the objectives proposed in the Application of 2nd August, 1966 and in the Semiannual Progress Report no. 1 forwarded in April, 1967 to The Council for Tobacco Research.

The observation of adrenal cortical damage in carcinogenesis by hydrazine/isonicotinic acid hydrazide (INH) seems a profitable line of investigation. In lung cancer patients changes in endocrine glands have been described. This is still the starting point for study on whether any internal factor exists in lung cancer.

The aims of this research, therefore, remain chiefly:

A. To study whether the adrenals are involved in spontaneous and INH and hydrazine sulphate (h.s.) induced pulmonary tumours in mice and

B. To study whether the thyroid is involved in spontaneous and INH and h.s. induced pulmonary tumours in mice.

6. Give a Brief Statement of your Working Hypothesis if altered or modified:

There have been no changes made to the working hypothesis.

10035467754

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

(SEE ATTACHED PAGES)

8. Additional Requirements:

Nil

10035462755

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

New personnel: Dr. Gaetano Giraldo (See biographical sketch attached)

10. Publications or Papers in Press resulting from the Project or closely related work

(SEE ATTACHED LIST)

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures:

A. Spontaneous and INH and h.s. induced pulmonary tumours in the mouse in relation to adrenal hormones.

a. Incidence of spontaneous and induced pulmonary tumours in susceptible and resistant mice.

There have been no changes made to these experiments, which have been described in the Application and in Progress Report no. 1.

b. Incidence of spontaneous pulmonary tumours in susceptible adrenalectomized mice into which the adrenals of resistant mice have been grafted, and vice versa.

At the beginning of this experiment (see Application and Progress Report no. 1) A/Lac/Se and C57BL/Cb/Se mice, respectively susceptible and resistant to lung tumour induction, were used.

To assure that the adrenals take in the host it is necessary to treat the test mice at birth with lymphocytes of the donor mouse.

The administration (intravenous or intraperitoneal) of lymphocytes between these two substrains, however, is badly tolerated: none of the C57BL/Cb/Se mice treated with A/Lac/Se lymphocytes lived beyond a few days; the same thing happened when A/Lac/Se mice were the tests and the C57/Cb/Se the donors. None of the technical procedures used was effective in preventing the death of the test mice. For this reason, the C57BL/Cb/Se mice were replaced by CBA/Cb/Se. The tolerance between CBA/Cb/Se and A/Lac/Se mice has proved good, provided the test mice are treated within a few hours of birth. CBA/Cb/Se mice are considered resistant to lung tumour induction and therefore may replace the C57BL/Cb/Se.

Several newborn mice of the two substrains have already been treated, and survival is good; at 8 weeks they will be adrenalectomized, and have the adrenals of the lymphocyte donor grafted into them; they will be examined at natural death. This has already been described in the Application and in Progress Report no. 1.

c. Pulmonary tumorigenesis by INH and h.s. in susceptible adrenalectomized mice into which the adrenals of resistant mice have been grafted, and vice versa.

These experiments will be carried out as described in the Application, with CBA/Cb/Se mice replacing the C57BL/Cb/Se.

1003546756

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

d. Incidence of spontaneous pulmonary tumours in intact and in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F_1 hybrid mice.

No change has been made to the experiment as described in the Application but, as mentioned in Progress Report no. 1, (BALB/c/Cb/Se x C57BL/Cb/Se)F_1 hybrid mice will also be used.

e. Incidence of spontaneous pulmonary tumours in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F_1 mice into which the adrenals of the parents have been grafted.

No changes: the experiments will be carried out as described in the Application. In these experiments as well (BALB/c/Cb/Se x C57BL/Cb/Se)F_1 hybrid mice will also be used.

f. Pulmonary tumorigenesis by INH and h.s. in intact and in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F_1 hybrid mice.

Also in these experiments, in addition to the (C57BL/Cb/Se x A/Lac/Se)F_1 hybrid mice (as shown in the Application), (BALB/c/Cb/Se x C57BL/Cb/Se)F_1 hybrid mice will be used (as shown in Progress Report no. 1).

g. Pulmonary tumorigenesis by INH and h.s. in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F_1 hybrid mice into which the adrenals of the parents have been grafted.

These experiments will be carried out as previously described in the Application; (BALB/c/Cb/Se x C57BL/Cb/Se)F_1 hybrid mice will be added.

If it seems of use, (CBA/Cb/Se x A/Lac/Se)F_1 hybrid mice will be added to the experiments described in paragraphs d,e,f,g.

These different types of hybrids will be used because they are the product of substrains of mice in each of which the spontaneous incidence and the susceptibility to lung tumour induction are different. If these characteristics in the adrenalectomized hybrid test mice are the same as in the parent donor of the adrenals, the influence of these glands in lung carcinogenesis could be hypothesized.

B. Spontaneous and INH and h.s. induced pulmonary tumours in the mouse in relation to thyroid hormones.

No changes made to the experiments as described in a,b,c,d of the Application.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

C. Additional experiments.

In addition to the original experimental design, investigations are being carried out on:

Mitotic cell activity in the mouse lung and immunological approaches of INH and h.s. induced lung tumours in mice.

a. Preliminary studies on the mitotic cell turnover in the mouse lung.

This experiment is being carried out with special fixation and staining techniques in lung of lung tumour induction susceptible (BALB/c/Cb/Se) and resistant (CBA/Cb/Se and C57BL/Cb/Se) mice, treated and non treated and in different hormonal states.

This should allow us to observe the early cell changes and the cells chiefly involved in the regenerative process. Differences between the three groups of mice - treated, non treated and in different hormonal states - could be of significant interest.

b. Immunological approaches to chemically induced lung tumours in mice.

(i) Attempts to show whether lung tumours obtained with INH and h.s. are merely accelerated spontaneous tumours or whether they are induced.

It has been shown that chemically induced tumours develop specific antigens, whereas spontaneous tumours do not.

Moreover, whereas virus induced tumours usually carry antigens specific to the virus and therefore common to every tumour induced by that virus, the tumour specific antigens of chemically induced tumours differ from tumour to tumour.

The application of this approach to INH and h.s. induced lung tumours could proceed along the following lines: part of a large lung adenoma could be transplanted into mice of the same strain; when it grew out, a part of it could be excised and fragments implanted into other mice, and removed when they started to grow out. The ability of further fragments of the same tumour, or of measured numbers of cells from it, to be rejected by the immunized mice would demonstrate the antigenicity of these tumours.

1003546758

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

This experiment will have to be repeated several times. It should, however, be confirmed also by other experiments, with the aim of showing that the participation of a virus in the development of the pulmonary tumour can be excluded, but this will not show that a hormonal factor is not involved. The latter is an internal component which probably does not act by itself but, in favourable conditions, might allow the action of INH or h.s. or of any other carcinogen.

(ii) Attempts to influence the growth of urethane induced primary lung tumours in BALB/c mice by stimulation of immunocompetent cells of the host.

Previous work done in this laboratory (1965, 1966) has shown that (the depression of immunocompetent cells obtained by) neonatal thymectomy potentiates the oncogenic effect of a single dose of urethane in BALB/c mice. In fact, in thymectomized mice lung tumours develop earlier, are more numerous, grow to a greater size, metastasize more frequently than in controls treated with the same dose of urethane, and they only rarely show foci of necrosis and/or fibrosis.

Theoretically, a stimulation of immunocompetent cells in mice treated with a single dose of urethane could reduce the oncogenic effect of the chemical compound. Following this approach, BALB/c mice inoculated with urethane will be treated with incomplete and complete Friend's adjuvant, with urethane-induced secondary BALB/c lung tumour grafts, and with a cell-free homogenate of the same tumour.

8. (See printed form)

9. Biographical Sketch of Dr. Gaetano Giraldo.

Title: Assistant scientist, Division of Cancer Research, University of Perugia

Place of Birth: Naples, Italy Present Citizenship: Italian
Sex: Male
Education and Experience: Dr. Giraldo graduated M.D. from the University of Naples in 1962. He is a staff member of the Institute of Pathological Anatomy and Division of Cancer Research, University of Perugia, from 1963. Dr. Giraldo has published 17 papers on various subjects.

1003546759

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

10. Publications or Papers in Press resulting from the Project or closely related work.

1. Ribacchi, R. and Giraldo, G.: Sui fattori intrinseci della cancerogenesi polmonare del topo: effetti della timectomia neonatale. Lav. Anat. Pat. Perugia 26, 127-136, 1966.

2. Severi, L. and Biancifiori, C.: Hepatic carcinogenesis in CBA/Cb/Se mice and Cb/Se rats by hydrazine sulphate. International Conference on Hepatic Regeneration, Montecatini, 29th-30th October, 1966. (In press)

3. Severi, L. and Biancifiori, C.: Hepatic carcinogenesis in CBA mice and Cb rats by isonicotinic acid hydrazide and hydrazine sulphate. Symposium on Hepatomas, Philadelphia, 19th-20th May, 1967. (The paper will be forwarded to the Journal of the National Cancer Institute for publication)

10035467 60

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year) (October 1st, 1967 to September 30th, 1968)

	% time	Amount
A. Salaries (Personnel by names or category)		
Professional		
Dr. Rodolfo Ribacchi	50%	R
Dr. Emilio Bucciarelli	50%	
Dr. Gaetano Giraldo	50%	
Technical		
Histological technician	100%	
Animal caretaker	100%	
Sub-Total		
$ — R		R
B. Consumable Supplies (list by categories)		
Animal food and sawdust $ 1,000		
Chemical drugs 500		
Staining materials 250		
Surgical instruments 150		
Glassware 100		
Sub-Total		
3,000		3,000
C. Other Expenses (itemize)		
Record cards, registers, labels		
Sub-Total		
300		300
D. Permanent Equipment (itemize)		
800 animal cages		1,700
		1,700
E. Overhead (15% of A+ B +C)		
Total		15,000
$ 11,600		

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature] Telephone
Director of Project

Signature [signature] Telephone
Business Officer of the Institution

10035476761

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Hormonal factor(s) in pulmonary carcinogenesis (by hydrazine). (Severi, L. and Biancifiori, C.)	The Anna Fuller Fund, New Haven, Connecticut	$ 5,000 per year	Dec. 1965 to Nov. 1968
Lung tumorigenesis by isoniazid (INH), its metabolite hydrazine sulphate and derivatives of hydrazine. (Biancifiori, C.)	The Council for Tobacco Research, 633 Third Avenue, New York N.Y. 10017	$11,100 10,100	Ap. 1966 to Mar. 1967 Mar. 1968

Pending

100354676?

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#569R1 MOSER

1003546763

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 3, 1967

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: A renewal application from Kenneth M. Moser, M.D. - NO. 569-R1.

We enclose herewith a renewal application from Dr. Kenneth M. Moser, of Georgetown University Medical Center, Washington, D.C.

The research program seems to be going forward according to plan, and since this request is for the second year of an original two-year plan, it is essentially routine.

Robert C. Hockett

100354676

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

No. 569-R1
Activated: 10/15/66
Cf. #398
Activated: 10/15/63
Renewed: 10/15/64
Renewed: 10/15/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application For Renewal of Research Grant

First ☒ Second ☐

Date: July 31, 1967

1. Name of Investigator(s): (include title and degrees) Kenneth M. Moser, M.D.
Associate Professor of Medicine

2. Institution & Address: Georgetown University Medical School, Washington, D. C. 20007

3. Short Title of Project: Smoker-Non-Smoker Differences in Activation of Fibrinolytic-Coagulation Systems.

4. Proposed Renewal Starting Date: (Anniversary or other) 1 November, 1967.

5. Discuss any Important Changes or Additions to Objectives or Specific Aims: No major changes have occurred in objectives or specific aims from those stated in the 1966 application. The goal is still to define smoker-non-smoker differences in coagulation-fibrinolytic behavior which might bear upon the statistic relationship between heart disease (especially coronary thrombosis) and cigarette smoking.

1003546765

6. Give a Brief Statement of your Working Hypothesis if altered or modified: The basic hypothesis remains unaltered; namely, that smoking may enhance thromboatherogenesis via the coagulation-fibrinolytic system.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages) At present, there have been no major changes in experimental design. However, if current trends in data are substantiated three additions to the protocol may be instituted: 1) study of the changes in "platelet adhesiveness" associated with smoking (in smokers) or hyperventilation (non-smokers); 2) limited studies of fibrinogen turnover rates in age-sex matched smokers and non-smokers; 3) addition of an exercise test to the nicotinic acid stimulus; i.e., to determine whether exercise produces the same type of smoker-non smoker differences as does nicotinic acid.

The rationale for these potential additions lies in the data appended (Tables I-3). We have recently subjected a considerable amount of our data in this study to computer statistical analysis (mean, standard deviation, standard error, correlation and "t" tests). This analysis has revealed several instructive smoker-non-smoker differences. In baseline-only studies (Table I) smokers have a statistically significant ($P < .05$) higher level of plasminogen. While other differences exist, they are not statistically significant. However, in this analysis there is a significant positive correlation between age and fibrinogen concentration in non-smokers - not in smokers. Thus smokers do not apper to have the expected age-related rise in fibrinogen, although the mean fibrinogen is higher in smokers. (Continued - pages 2-A thru 2-E)

8. Additional Requirements: We currently have no major additional requirements.

10035466766

9. Changes in Personnel with Biographical Sketches of new Personnel (append) Dr. Paul G. Harsanyi joined our research staff this year. (see attached bibliographic sketch, page 2-F)

10. Publications or Papers in Press resulting from the Project or closely related work The data presented in Tables I-3 is currently being prepared for publication.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions, cont.

On the acute smoking (or hyperventilation) studies, other interesting data appear (Table 2). Again, there is a positive age-fibrinogen correlation in non-smokers only, with fibrinogen levels again higher (but not quite significant at $P < .05$) in smokers. The plasminogen levels are again significantly higher in the smokers. In both broups, there is a highly significant rise in plasminogen ($P < .001$) with smoking (or hyperventilation). Partial thromboplastin time behavior is also interesting. In this study, baseline PTT was longer in smokers (not significantly) but fell with smoking and after smoking. In non-smokers, PTT increased slightly with and following hyperventilation.

In the nicotinic acid study (Table 3) to date, instructive data have been generated. The most striking is the fact that nicotinic acid provokes a much greater (and apparently more sustained) enhancement of activator activity in the non-smokers than in the smokers. Significant differences (as measured by both ELT and A-P plates) exist between smokers and non-smokers at all intervals. Thus, the non-smoker seems to release activator more readily than the smoker. If this is true under all circumstances, it would suggest thrombolysis may be impaired in smokers in terms of its response to a given stress.

As the data are extended, further computer analyses will be carried out. (A program has been developed for this specific purpose). Analysis of smoker-non-smoker differences between different age and sex-matched groups are contemplated. These may reveal differences obscured by combining all data, as has been done in the analysis reported in Tables 1-3.

1003546767

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

	Smokers (60)	Non-Smokers (50)
Age	40.1 ± 14.8	39.7 ± 21.0
Pack-Years	18.0 ± 15.4	0
Pro. T. (Sec)	17.9 ± 3.1	17.0 ± 2.1
P.T.T. (Sec)	89.3 ± 30.7	81.8 ± 32.6
ELT (Min)	234.0 ± 74.0	238.0 ± 72.0
Fibrinogen (mgm%)	451.0 ± 147.0	409.0 ± 145.0
A-P (mm^2)	192.0 ± 121.0	178.0 ± 129.0
P'gen (mm^2)	329.0 ± 70.0	297.0 ± 94.0
C. P'gen (CRU)	5.6 ± 4.5	3.7 ± 3.3

Table One: Smokers versus non-smokers. Fasting morning values.

1003546768

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Smokers (50)

	Baseline	Smoking	Post-Smoking (60')
Age	41.0 ± 15.5	--	--
Pack-Years	19.8 ± 15.6	--	--
P. T.	17.2 ± 2.8	17.2 ± 2.8	17.2 ± 3.0
P.T.T.	96.3 ± 38.1	88.8 ± 33.8	85.9 ± 32.3
ELT	241.0 ± 76.0	229.0 ± 75.0	242.0 ± 72.0
Fibrogen	451.0 ± 156.0	451.0 ± 151.0	443.0 ± 148.0
A-P	196.0 ± 126.0	206.0 ± 123.0	191.0 ± 117.0
P'gen	307.0 ± 73.0	314.0 ± 81.0	307.0 ± 72.0
C. P'gen	5.5 ± 5.0	13.4 ± 5.4	7.5 ± 6.0

Non-Smokers (40)

Age	41.5 ± 21.2	--	--
Pack-Years	0	--	--
P. T.	16.6 ± 2.1	17.0 ± 2.4	16.9 ± 2.3
P.T.T.	82.9 ± 21.7	92.6 ± 46.7	85.7 ± 49.9
ELT	243.0 ± 64.0	250.0 ± 60.0	240.0 ± 69.0
Fibrinogen	411.0 ± 140.0	404.0 ± 139.0	402.0 ± 149.0
A-P	172.0 ± 74.0	183.0 ± 88.0	196.0 ± 85.0
P'gen	288.0 ± 60.0	285.0 ± 69.0	290.0 ± 60.0
C. P'gen	3.8 ± 3.9	11.9 ± 3.4	4.6 ± 3.6

Table Two: Smokers and non-smokers studied before, at completion and 60 minutes after completion of a 30 minute smoking or mild hyperventilation period.

1003546769

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Smokers

	0	10	20	40	60
Age	37.8 ± 12.8	--	--	--	--
Pack-Years	16.3 ± 9.8	--	--	--	--
P. T.	16.0 ± 1.9	16.4 ± 2.4	16.4 ± 2.2	16.3 ± 1.8	16.1 ± 1.8
P.T.T.	90.3 ± 35.7	97.8 ± 42.7	102.0 ± 45.0	98.9 ± 44.0	86.1 ± 55.9
ELT	263.0 ± 60.0	151.0 ± 112.0	147.0 ± 111.0	178.0 ± 96.0	199.0 ± 90.0
Fibrinogen	419.0 ± 126.0	408.0 ± 156.0	272.0 ± 77.0	419.0 ± 138.0	439.0 ± 145.0
A-P	135.0 ± 142.0	345.0 ± 277.0	354.0 ± 279.0	271.0 ± 220.0	233.0 ± 180.0
P'gen	262.0 ± 79.0	266.0 ± 77.0	265.0 ± 73.0	274.0 ± 61.0	275.0 ± 75.0
C. P'gen	3.8 ± 2.4	4.4 ± 2.6	4.4 ± 2.8	4.2 ± 6.6	3.9 ± 2.2

Table Three: (A) Smokers (38) and non-smokers (30) studied before and at 10, 20, 40, and 60 minutes after 100 mgm nicotinic acid given intravenously.

1003546770

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Non-Smokers

	0	10	20	40	60
Age	34.9 ± 12.8	---	--	--	--
Pack-Years	0	--	--	--	--
P. T.	16.4 ± 2.0	16.4 ± 2.3	16.2 ± 2.2	16.3 ± 2.2	16.6 ± 2.3
P.T.T.	87.6 ± 32.9	82.9 ± 31.6	87.0 ± 37.8	87.5 ± 39.0	85.2 ± 46.8
ELT	223.0 ± 73.0	74.0 ± 85.0	124.0 ± 120.0	102.0 ± 94.0	143.0 ± 91.0
Fibrinogen	355.0 ± 126.0	274.0 ± 159.0	316.0 ± 98.0	314.0 ± 153.0	360.0 ± 130.0
A-P	167.0 ± 109.0	508.0 ± 288.0	--	--	--
P'gen	280.0 ± 83.0	264.0 ± 78.0	--	265.0 ± 73.0	266.0 ± 71.0
C. P'gen	3.3 ± 1.9	4.2 ± 2.6	3.3 ± 2.3	3.6 ± 2.0	3.3 ± 1.9

Table Three: (B) Smokers (38) and non-smokers (30) studied before and at 10, 20, 40, and 60 minutes after 100 mgm nicotinic acid given intravenously.

1003546771

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Page 2, Item 9: Personnel with Biographical Sketches: Dr. Paul G. Harsanyi.

Date of birth: R R
Place of birth: Budapest, Hungary
Marital status: R R
M.D.: University of Budapest, 1962.
Internship: University of Budapest Medical Center, 1962-62.
Residency: University of Pecs Medical Center, 1963-66.
Visiting Research Scientist: University of Vienna Medical School, 1966.
Research Associate: Georgetown University Medical School, 1966 to present.

(NOTE: Dr. Harsanyi, who joined our Program in 1966, has made a considerable contribution to our research efforts. His departure from Hungary interrupted a promising research career there. We are delighted he has been able to resume it with us.)

1003546722

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

11. Budget (for coming year)

	% time	Amount
A. Salaries (Personnel by names or category)		
Professional		
Kenneth M. Moser, M. D.	15	
George C. Hajjar, M. D.	50	
Paul G. Harsanyi, M. D.	50	
Technical		
Charles Brunswick, B.S.	100	
Dorothy Brown	100	
Johnnie Burbank	50	0.00
Sub-Total		R
Fringe Benefits (7.12%)		748.00=11,248.00
B. Consumable Supplies (list by categories)		
Reagents, solutions		500.00
Glassware, needles, miscellaneous		600.00
Sub-Total		1,100.00
C. Other Expenses (itemize)		
Equipment maintenance and repair		300.00
Preparation of slides, graphs		200.00
Data analysis (computer time)		300.00
Payments to volunteers		400.00
Sub-Total		1,200.00
D. Permanent Equipment (itemize)		
Double Vortex Mixer		200.00
Hand Calculator		200.00
		400.00
E. Overhead (15% of A+ B +C)		2,032.00
Total		15,980.00

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Kenneth M. Moser
Director of Project

Signature Doris J. Roth
Business Officer of the Institution

Telephone 337-3300 Ext. 318

Telephone

10035492/23

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Role of Fibrinolysis in Pulmonary Embolism	Bezalel and Breth Foundations	15,000/year	1967-68
Pending	Control Mechanisms of the Fibrinolytic System in Man	National Institutes of Health	25,000/year	Jan. 1, 1968 to Dec. 31, 197

1003546774

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#573R1 LOOSLI

1003546775

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 15, 1967

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Lynch, Dr. Reimann and Dr. Sommers.

FROM: Robert C. Hockett

SUBJECT: First renewal application from Clayton G. Loosli, M.D. - No. 573R1.

We enclose herewith the first renewal application of Dr. Clayton G. Loosli of the University of Southern California School of Medicine.

This represents the second year of a three-year program that was activated on October 1, 1967 and promised priority in consideration for the second and third years.

A detailed Progress Report accompanies the application (copy attached).

R. C. H.

1003546276

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

VIRUS - PREVIOUS INFECTION

NO. 573R1
Activated: 10/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Lynch
Dr. Reimann
Dr. Sommers

633 THIRD AVENUE
NEW YORK, N. Y. 10017

Application For Renewal of Research Grant

First ☒ Second ☐

Date: August 7, 1967

1. Name of Investigator(s): (include title and degrees) Clayton G. Loosli, Ph.D., M.D.
Hastings Professor of Medicine and Pathology, USC School of Medicine
2025 Zonal Avenue, Los Angeles, California 90033

2. Institution & Address:
The Attending Staff Association
Los Angeles County General Hospital
1200 North State Street
Los Angeles, California 90033

3. Short Title of Project: LUNG TISSUE REACTIONS TO AIRBORNE CHEMICAL AND BIOLOGICAL AGENTS

4. Proposed Renewal Starting Date: (Anniversary or other) October 1, 1967

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

The objectives are the same. This past year the pathogenesis of lung changes in mice exposed to atmospheres of NO_2 in concentrations of 10, 20, 40 ppm have been made. Most of our studies have been made on animals exposed to 38 to 42 ppm over periods from 1 day to 8 weeks, after which the mice began to die. The rapidity of development of lung lesions is directly related to the concentration of NO_2 in the air. In order to observe what the lung changes were like in the final stages before death, the higher concentration was chosen. This coming year, with the availability of several separate exposure chambers, comparative studies will be made of the lungs of animals exposed to the three concentrations (10, 20, 40 ppm of NO_2) by histological, histochemical, biochemical and electronmicroscopic procedures.

Studies of the lungs and other organs (liver, spleen, kidney, adrenal, brain, heart) of mice exposed to different concentrations of SO_2 will be continued. Studies of the pathogenesis of PR8 influenza A virus infections in NO_2 and SO_2 exposed animals will be continued. An attempt will be made to elucidate the mechanism(s) of increased resistance of NO_2 exposed mice to influenza A infections. The pathogenesis of PR8 influenza A airborne virus infections in SO_2 mice will be studied to determine if they, as do NO_2 exposed mice, develop a resistance.

The susceptibility of NO_2 and SO_2 exposed mice to airborne staphylococcus aureus will be continued and, in addition, the pneumococcus and hemophilus influenza organisms will be employed.

6. Give a Brief Statement of your Working Hypothesis if altered or modified: There is no change in our working hypothesis. Our aim is to define the lesions due to NO_2 and SO_2 exposure, the lesions due to bacteria and influenza A virus, and the effect of super imposition of these lesions in the same animal.

1003549777

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

There is no basic change in the experimental design and procedures. A full complement of skilled and experienced technical personnel have been procured in histology, biochemistry, histochemistry, bacteriology, virology and electronmicroscopy. I believe the great importance of our study rests in the simultaneous study of the histological, biochemical and histochemical changes in the lungs and other organs of mice subjected to NO_2 and SO_2 exposure alone and in combination with exposure to the above named viruses and bacteria.

This past year oxygen consumption, alkaline and acid phosphatase and lactic dehydrogenase measurements have been made in NO_2 exposed animals. This coming year glycolysis, lipid metabolism and antitrypsin activity will be measured in the lungs of NO_2 exposed animals, as well as urinary steroids and transaminase in the serum.

8. Additional Requirements:

Generally, all the facilities to carry on the above studies are available. The output can be greatly increased by having several chambers in which to expose mice to different concentrations of NO_2 and SO_2 for different periods of time. This will allow us to make short term observations while allowing other mice to be observed for longer periods of time at low levels of NO2 and SO_2. It is estimated that very satisfactory chambers can be constructed for $500 each, including fans, flexible exhaust piping, etc.

Also, a drying oven is needed in the biochemistry section to facilitate the analysis of tissues.

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

There are no changes in personnel. Ramon D. Buckley, Ph.D., Assistant Professor of Biochemistry, directs the biochemical studies, and is co-director with salary support from the NIH grant. Between the two grants he devotes 80 percent of his time to the studies. The technical personnel are all experienced.

10. Publications or Papers in Press resulting from the Project or closely related work

No papers have been published or are in press as yet from studies supported by this grant.

10035467728

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0089

11. Budget (for coming year)

A. Salaries (Personnel by names or category)	% time	Amount
Professional		
Clayton G. Loosli, M.D., Ph.D.	50	None
*Ramon D. Buckley, Ph.D.	75	None
Technical		
*Sylvia Moss, M.S., Histol-Biochemist	100	None
*Elizabeth Vinceguerra, B.S., Biochemist	100	None
*Johnnie Bilbrew, Jr., Animal Caretaker	100	None
Mary Bresnahan, B.S., Virologist	100	$7,560
John Hardy, B.S., Electronmicroscopist	100	7,560
Ernestine Pruiet, B.S., Bacteriologist	100	7,119
Edna M. Stone, M.S., Histology-Histochem. Technician	100	8,820
Fringe Benefits, 8%		2,485
	Sub-Total	$33,544

*Paid from NIH grant (see page 4)

B. Consumable Supplies (list by categories)		
Mice and rabbits	$ 2,000	
Supplies, histol., bacteriol. & virol.	2,500	
Gen.&spec.chem.reagents,enzymes,cofactors	1,000	
Glassware	500	
Dry ice, water de-ionizers, etc.	1,000	7,000
	Sub-Total	7,000

C. Other Expenses (itemize)		
Travel for Principal Investigator and Dr. Buckley	$ 750	
Service, Spinco, spectrophotometers, etc.	1,000	
Repairs on equipment, office supplies, publication costs, misc. supplies	1,250	
	Sub-Total	3,000

D. Permanent Equipment (itemize)	
Construction of three exposure chambers with motors, fans, etc.	1,500
E. Overhead (15% of A + B + C) 15% of $43,544	6,532
Total	51,576

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Clayton H. Loosli M.D.
Director of Project
225-3115, Ext. 2175
225-1511, Ext. 203
Telephone

Signature J. S. Gott
Business Officer of the Institution
J. S. Gott
Business Manager
225-3115
Ext. 3826
Telephone

10035467729

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

urrent

Title of Project	Source	Amount	Duration
Lung Tissue Reactions to Chemical and Biological Agents	USPHS, NIH, NIAID A1 AP 07782-01	$41,970	Jan-Dec 1967
		39,700	1968
		39,700	1969
		$121,370	

nding

1003546780

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

SUPPLEMENTS

10035467781

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#456R2—HOMBURGER

1003546782

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. July 25, 1967

TO: The committee comprising Drs. Little, Chm., Dr. Jacobson, Dr. Sommers and Dr. Reimann.

FROM: Robert C. Hockett

SUBJECT: Request for a supplementary grant from Freddy Homburger, M.D. - No. 456R2.

We enclose herewith a letter from Dr. Freddy Homburger of Bio-Research Consultants, Inc., requesting additional funds.

Payment for the additional smoking machines and the cost of the patent, mentioned in the first two paragraphs of the letter, have already been approved.

The request for a consultant fee of $6,000.00 for Mr. Walton beginning April 1, 1967 is at the same rate as we provided for the previous year.

R. C. H.

1003546783

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

No. 456R2
Activated: 1/1/65
Renewed: 1/1/66
Renewed: 1/1/67

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Little, Chm.
Dr. Jacobson
Dr. Sommers
Dr. Reimann

Application For Research Grant

Date: JUNE 19, 1967

1. Name of Investigator(s): (include Title and Degrees) FREDDY HOMBURGER, M.D.
President and Director

2. Institution & Address: BIO-RESEARCH CONSULTANTS, INC.
9 Commercial Avenue
Cambridge 41, Massachusetts

Dear Mr. Hoyt:

In reply to your letter of June 16th, I wish to thank you for formalizing the contract to purchase from Mr. Richard Walton six additional machines at $ 1,495. each, and we shall be prepared to inspect and test these machines prior to shipping them, at your direction, to other investigators.

We also have passed on to Mr. Walton your decision to pay for the patent for the 'Walton machine' approximately $ 500. with the understanding that assignment of the patent will be made to The Council for Tobacco Research or its designee.

Further to follow up on your letter, this is to request formally that the Scientific Advisory Board of The Council approve an additional consultant fee for Mr. Walton of $ 6,000. for one year beginning April 1st, 1967, which will be paid to Mr. Walton quarterly for his participation in our development of a 'Walton smoking machine' for the preparation of cigarette smoke condensates and for his continued consultation in our adaptation of the present 'Walton smoking machine' to studies on other species, and in the adaptation of the 'Walton smoking machine' to the exposure of organs other than the respiratory tract.

We are submitting this request to you retroactive to April 1st, 1967 because Mr. Walton has continued since that date and will continue for another year from that date to be helpful to us in the matters referred to above.

We appreciate your most helpful cooperation.

Yours sincerely,

Freddy Homburger, M.D.
President and Director

FH:er

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546784

#467R2-S WESTFALL

1003546785

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. August 28, 1967

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Cattell, Dr. Jacobson and Dr. Lynch.

FROM: Robert C. Hockett

SUBJECT: Request for Supplementary grant from Thomas C. Westfall, A.B., M.S., Ph.D. - No. 467R2-S.

We enclose herewith a request for a supplement grant from Dr. Thomas C. Westfall of the University of Virginia School of Medicine.

The Board has already approved (May 20, 1967) a grant of $11,500.00 effective on September 1, 1967 to complete the three-year research plan that Dr. Westfall undertook beginning September 1, 1965. On June 1st he requested permission to reallocate $3,000 from the present year's budget toward purchase of a liquid scintillation spectrometer and was given staff permission to do so, subject to ratification by the Board at their next meeting.

The present application for an additional appropriation is quite detailed and is distributed for consideration on its merits at the forthcoming meeting.

R. C. H.

1003549786

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

NO. 467-R2-SUP.
Activated: 9/1/65
Renewed: 9/1/66
Renewed: 9/1/67

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Bing, Chm.
Dr. Cattell
Dr. Jacobson
Dr. Lynch

Application For Research Grant

Date: August 24, 1967

1. Name of Investigator(s): (include Title and Degrees)

Thomas C. Westfall, A.B., M.S., Ph.D.

2. Institution & Address:

Department of Pharmacology
University of Virginia School of Medicine
Charlottesville, Virginia 22901

3. Short Title of Project: Supplemental Grant Request

Funds to aid in the purchase of a Scintillation Spectrometer (to supplement grant entitled, "Action of Nicotine on Subcellular Distribution of Catecholamines in Brain and Heart")

4. Proposed Starting Date:

October 1, 1967

5. Anticipated Duration of this Specific Study:

on separate sheet

6. Brief Descripton of Objectives or Specific Aims:

on separate sheet

7. Give a Brief Statement of your Working Hypothesis:

on separate sheet

1003546787

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

on separate sheets

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

on separate sheet

10. Additional Requirements:

none

11. Biographical sketches of all principal and professional personnel (append)

see appended curriculum vitae

12. List of publications: (Five most recent as pertinent) (append)

see appended curriculum vitae

10035496788

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Technical		
Sub-Total		
B. Consumable Supplies (list by categories)		
Sub-Total		
C. Other Expenses (itemize)		
Sub-Total		
D. Permanent Equipment (itemize)		7,500
To aid in the purchase of a Packard Automatic Tri Carb Scintillation Spectrometer		
E. Overhead (15% of A+B+C)		
Total		7,500

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Thomas C. Westfall 2014
Director of Project

Telephone

Signature MR Kuhlman 3225
Business Officer of the Institution

Telephone

10035462789

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Catecholamine Metabolism Following Cigarette Smoking and Nicotine Administration	The American Medical Association Education and Research Foundation Committee for Research on Tobacco and Health	9218.	Jan. 1, 1967 to Dec. 31, 1967
Pending				

100354679O

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

5. Duration of Specific Study

This equipment would be used to help complete the project listed above which is currently being sponsored by The Council. In addition, it is anticipated that this instrument will be used in many future projects under contemplation by the principle investigator.

6. Importance of a Scintillation Spectrometer to the Specific Aims and Objectives of the Principle Investigator.

The purpose of this supplemental grant application is to request funds which will aid in the purchase of a scintillation spectrometer. This instrument is greatly needed in achieving the following overall specific aims.

1) To ascertain the importance of certain biogenic amines in helping to explain the central and peripheral actions of nicotine.

2) To specifically determine if there is an adrenergic mechanism involved in the central action of nicotine.

3) To specifically determine whether or not nicotine is exerting any of its peripheral action by the release of norepinephrine directly from adrenergic nerve terminals.

CENTRAL ADRENERGIC ACTIONS OF NICOTINE

Attempts at determining whether or not there is an adrenergic mechanism involved in the central action of nicotine are currently being carried out in this laboratory by studying the effect of nicotine on the subcellular distribution of certain amines in specific brain regions where these amines are present in large concentrations. Studies have been made on brain areas containing large concentrations in an attempt to prevent a masking of a possible effect that might be taking place due to tissue which has very little or no amine content. Our results, to date, indicate that there are certain changes in the level of these amines in various regions of the central nervous system (Westfall, et al., 1967). These studies are limited, however, to measurement of changes in the static storage levels of catecholamines and other amines in the brain. In addition, they have not included areas such as the cerebellum which contains very little endogenous norepinephrine but may be very important because of the active metabolism of amines recently observed to be taking place there.

1003549791

Although these above mentioned studies can produce important results and can give us some ideas of a release of amines by nicotine, they tell us nothing about the dynamic aspects of metabolism of amines in the brain.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

We are becoming more and more aware that the storage levels of norepinephrine, dopamine and serotonin are not static but reflect dynamic equilibria between the rates of formation and the rates of utilization of the amines (see reviews by Glowinski and Baldessarini, 1966; Hornykiewicz, 1966). In other words, there is a constant amount of norepinephrine in nerve endings because a continuous synthesis or input is balanced by a continuous efflux onto receptors or metabolizing enzymes. The level of norepinephrine will be altered, therefore, if one of these rates has been changed. Numerous investigators have emphasized that synthesis, storage release and metabolism are not unrelated phenomena and should be considered together in interpreting drug action (Neff, et al., 1965; Costa, et al., 1966). Therefore, it may be that changes in the turnover rate of these amines are a better measure of drug action than mere changes in the concentrations of the amines which could remain constant or even decline despite an increased rate of synthesis.

Recent studies by Iversen and Glowinski (1966), and Glowinski and Iversen (1966a,b) have shown that different turnover rates of norepinephrine occur in several different regions of the rat brain. These investigators observed that the brain regions seemed to fall into three classes. The cerebellum appears to have the fastest turnover of norepinephrine, with a half-life of about 2 hours. Although this brain region has such a fast turnover, it is quite interesting that this area has a very low endogenous norepinephrine content. This would seem to indicate that the small stores of norepinephrine are in an unusually active metabolic state. This rapid turnover may even be correlated in some way with its ability to form adenosine 3',5'-phosphate. Along with the low endogenous norepinephrine content, the cerebellum seems to accumulate very little H^3-norepinephrine. On the other hand, the cerebellum appears to accumulate more H^3-metabolites of norepinephrine when compared to brain regions with slower turnover times.

Following the cerebellum, the brain regions with the next fastest turnover rates seem to be the cortex and hippocampus with half-lives of about 3 hours. The regions with the slowest rates of turnover of norepinephrine are the hypothalamus and the medulla oblongata with half-lives of 4 hours. Interestingly, these same regions have the highest concentration of endogenous norepinephrine and seem to accumulate the greatest amounts of H^3-norepinephrine and the least amount of H^3-metabolites. These experiments point out the important fact that an inverse relationship between turnover and endogenous concentrations of norepinephrine in various parts of the brain seem to exist. This probably indicates that areas with small endogenous levels actually have a faster active metabolism of catecholamines than regions containing higher levels of norepinephrine. These observations emphasize, therefore, the importance of studying turnover rates rather than only measuring steady state levels of catecholamines in various brain regions following drug administration.

At the present time there appears to be three available methods for measuring the rate of turnover of norepinephrine in the brain. Two of these methods involve the use of labelled techniques. The first method measures catecholamine turnover by introducing

10035467792

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

labelled norepinephrine into the lateral ventricle and follows the changes in specific radioactivity with time. The exponential decline of H^3-norepinephrine that occurs is interpreted as the turning over of the endogenous norepinephrine stores at a constant rate, by the continual synthesis of norepinephrine and removal of an equivalent amount of labelled and unlabelled amine. The slope of the exponential decline is a reflection of the turnover rate and, therefore, the rate of synthesis of endogenous norepinephrine. The second is by using labelled precursors such as tyrosine, dopa or dopamine and measuring the amount of formed norepinephrine from these precursors. The final method is by inhibiting catecholamine biosynthesis with such drugs as α-methyl-p-tyrosine. When synthesis is blocked, the rate of disappearance of endogenous catecholamines from peripheral and central stores gives a measure of the rate of utilization of amine stores.

Of these three methods of measuring catecholamine turnover in the brain, the use of exogenous tritiated norepinephrine appears to have distinct advantages. The use of α-methyl-p-tyrosine, for instance, is very limited by the difficulty of measuring very low concentrations of endogenous norepinephrine in discrete brain areas with the chemical techniques available. The use of H^3-dopamine or other precursors is an improvement over the previous method but may be complicated by the possibility that a prolonged synthesis of H^3-norepinephrine may occur in the presence of persistant amounts of the labelled precursor. The available evidence, however, seems to suggest that this fact does not occur to any important extent with H^3-dopamine (Iversen and Glowinski, 1966).

It can be concluded, therefore, that equipment necessary to measure labelled substances is required to adequately measure turnover of catecholamines in the brain in response to nicotine. These studies would give a direct measurement of the influence of nicotine on the dynamic metabolism of catecholamines in the central nervous system. This seems particularly important for regions such as the cerebellum which has been demonstrated to have a rapid metabolism but which has not been studied even for changes in static catecholamine levels because of the technical problems in measuring the low levels (see Westfall, et al., 1967) present there.

1003546793

PERIPHERAL ADRENERGIC ACTIONS OF NICOTINE

Another important use of this instrument will be its application in studies aimed at further evaluating the role of nicotine on peripheral amine stores in adrenergic nerve terminals. As in the central nervous system, there is likewise a dynamic equilibria between the rates of formation and rates of utilization of catecholamines in peripheral sympathetic nervous structures. Therefore, it is equally important to study the influence of nicotine on the turnover of norepinephrine in adrenergically innervated organs such as the heart and spleen. Similar to different brain regions, there are differences in the turnover rate of norepinephrine in various organs as well (Burack and Draskoczy, 1964).

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Previous publications from this laboratory have pointed out the inconsistencies and controversies that exist in the literature concerning the ability of nicotine to release norepinephrine from storage sites in adrenergically innervated organs (Westfall, 1965a,b; Westfall, et al., 1967). One of the main reasons for these discrepancies is probably a result of the difficulty in measuring and detecting the small changes in endogenous amine that are taking place following the administration of nicotine. A more convenient method for studying cardiac norepinephrine in the rat has been used by Hertting and co-workers (1961) and by Daly, et al., in the mouse (1966). In both cases they have shown that following tracer doses of labelled norepinephrine the amines are taken up into the heart, equilibrate with endogenous amine and most important are affected in a similar manner by agents that cause or inhibit norepinephrine release. This method has been described as being rapid, simple, economical and quite reliable. Other methods have been described using a perfused heart preparation (Nash, et al., 1967). It is anticipated that similar methods would be very valuable and useful in studying the influence of nicotine on norepinephrine stores. These methods appear even more valuable when it is realized that drugs can act by releasing norepinephrine from nerve endings without an appreciable change in the endogenous level as measured by the currently available biochemical methods. Because of the dynamic equilibria that exists between formation and utilization, any release of amine may be quickly replaced by synthesis and/or reuptake into nerve terminals. With the use of labelled amines described above, such a release can be much more easily detected.

Another potential use of the scintillation spectrometer would be to study the influence of nicotine on the uptake of norepinephrine into adrenergic nerves. Such a possible effect is suggested by the data of Nedergaard, et al. (1966). These investigators have reported that nicotine can potentiate the response of vascular smooth muscle to nerve stimulation by up to 60%. The same doses of nicotine occasionally produced small increases in resting tone. Similar types of potentiation of sympathetic effects exist for a large number of drugs such as cocaine or imipramine which are well known to block uptake or reuptake of norepinephrine into adrenergic nerve terminals (Carlsson, 1966; Trendelenburg, 1966). Therefore, it is quite possible that nicotine also possesses some ability to antagonize the uptake of norepinephrine into adrenergic nerve terminals. Such a possibility certainly deserves investigation.

7. Working Hypothesis.

1003546794

The synthesis, storage, release and metabolism of biogenic amines are not unrelated phenomenon and should be considered together in interpreting the action of nicotine in the central and peripheral nervous system. Changes in the turnover rate of amines such as norepinephrine will give a much clearer picture of the effect of nicotine on the dynamic metabolism of amines in brain and peripheral organs.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experiments Utilizing a Scintillation Spectrometer.

TURNOVER OF CATECHOLAMINES IN THE CENTRAL NERVOUS SYSTEM BEFORE AND AFTER NICOTINE ADMINISTRATION

As mentioned above, there are three available methods for studying the turnover of catecholamines in the brain at the present time. Of these three methods, the one which seems to offer the most advantages is the one which follows the changes in specific activity of exogenously administered tritiated norepinephrine.

The labelled amine will be injected into the lateral ventricle of rats anesthetized lightly with pentobarbital, using the recently described method of Nobel, et al. (1967). Nicotine in various doses will be administered prior to or following the intraventricular injection of DL-H^3-norepinephrine. The rats will be killed by decapitation at various times after the injection of norepinephrine and brains quickly removed, blotted and chilled. Seven regions will be dissected out on an ice cooled glass plate including the following.

a) cerebellum
b) medulla oblongata
c) hypothalamus
d) midbrain
e) striatum
f) hippocampus
g) cortex

Complete details of these dissections are given in the report by Glowinski and Iversen (1966). In addition, the plates in "Craigie's Neuroanatomy of the Rat" (Zeman and Innes, 1963) will be closely followed.

The tissues will be quickly weighed and homogenized in 10 to 15 ml of cold 0.4 N perchloric acid in a Ultra-turrax homogenizer. The homogenates will then be centrifuged at 10,000 x g for 10 min. After the addition of 0.1 ml of 1% disodium ethylenediamine-tetraacetate (EDTA) and 0.1 ml of a freshly prepared 1% solution of ascorbic acid, the pH will be adjusted to 8.3 - 8.5, with N normal NaOH. The sample will then be passed through a column containing aluminum oxide to remove the free catecholamines while the combined effluent and washing will be used for the estimation of the o-methylated metabolite, normetanephrine. Elution of the catecholamines from the alumina column will be carried out by the addition of 0.2 N HCl. An aliquot of this eluate will be used to determine the radioactivity of the free norepinephrine, while the tritiated deaminated metabolites will be extracted from the alumina eluates according to the method of Kopin, et al. (1961). Briefly, this involves taking an aliquot, acidifying it with 6 N HCl and saturating the solution with NaCl. The nonamine catechols are then extracted into ethylacetate. After centrifugation, an aliquot of the organic phase will be evaporated in a current of air

10035467795

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

in a glass vial and the radioactivity determined.

The o-methylated metabolite, normetanephrine will be assayed by the method described by Iversen, et al. (1966). Briefly, the effluent and washings from the alumina column used to extract the catecholamines will be adjusted to pH 6.5 and passed through a column (6 x 20 mm) of Dowex 50W-X4 in the sodium form. The resin will then be washed with glass distilled water, and the normetanephrine eluted with a mixture of equal quarts of 6 N hydrochloric acid and ethanol. Aliquots of this eluate will then be evaporated to dryness in vials.

The free catecholamines, normetanephrine and the deaminated metabolites will be assayed in a scintillation spectrometer after the addition of 4 ml ethanol and 10 quantities of 0.4% 2,5-diphenyloxazole and 0.005% 1,4-di(2-5-phenyloxazole)benzene in toluene. Tritiated o-methylated, deaminated metabolites can be estimated by the difference between total radioactivity of tissue extracts and the sum of (H^3) norepinephrine and other metabolites. A flow chart, enclosed in this report, gives a quick overall picture of the extraction procedures.

In some experiments, various subcellular constituents of the various brain regions will be assayed for their free catecholamine and metabolite content. In these experiments, the tissues will be initially homogenized in 0.3 M sucrose. One-fourth of the sample will be taken for assay of total radioactivity and the remaining extract spun at a low speed (10,000 x g - 10 min) to remove the coarse fraction. The low speed supernatant will then be spun at 100,000 x g for 40 min, yielding a supernatant and particulate fraction. The low speed sediment, high speed supernatant, high speed particulate and total tissue samples will then be extracted in 0.4 N perchloric acid and extracted according to the procedure described above for the differentiation of free, deaminated, o-methylated and deaminated o-methylated amines.

STUDY OF THE RELEASE OF NOREPINEPHRINE FROM ADRENERGIC NERVES BY NICOTINE

As has been mentioned in the previous section, controversies still exist as to whether or not nicotine releases appreciable quantities of norepinephrine from adrenergic nerves. Most of this controversy exists as a result of discrepancies in the literature because of the difficulty of available chemical methods for determining the small amounts of norepinephrine that might be released.

In the present studies, the influence of nicotine on the release of norepinephrine will be studied by measuring the amount of labelled norepinephrine remaining in the heart at a given period of time, following the prelabelling of the norepinephrine stores.

10035496296

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

These studies can be carried out in both mice and rats. For mice, a 0.1 ml solution (isotonic sodium chloride, containing 50 mg of heparin/l) of 1.0 mμ mole (5μ curies) of tritiated norepinephrine will be injected into the tail vein of male white mice. Nicotine in various doses will then be administered subcutaneously one hour later. The mice will then be sacrificed after 3 hours by a blow on the heads and the hearts quickly removed. The hearts will be immediately exsanguinated and homogenized in 10 vol of 0.4 N perchloric acid in an Ultra-turrax apparatus. Following homogenization, the extract will be centrifuged (10,000 x g for 10 min) and the supernatants taken for assay in a scintillation spectrometer, similar to the method described above. Endogenous norepinephrine will be simultaneously assayed according to the method of von Euler and Lishajko (1961).

In the studies on rats a similar procedure will be followed except that about 10 μC of tritiated norepinephrine will be administered by a tail vein and the animals sacrificed by decapitation.

In some experiments the influence of nicotine on the subcellular distribution of the labelled norepinephrine will be studied. In these experiments, the tissues will be homogenized in ice cold potassium phosphate buffer, pH 7.5. An aliquot will be taken and extracted in 10% trichloroacetic acid for assay of total heart. The remaining homogenate will be spun in a refrigerated centrifuge at a slow speed (10,000 x g, for 10 min). The slow speed supernatant will then be spun at 100,000 x g for 45 min in a ultracentrifuge. All fractions resulting from the differential centrifugation will then be taken for assay in a scintillation counter or adsorbed on alumina for endogenous norepinephrine determination following extractions in 10% trichloroacetic acid.

INFLUENCE OF NICOTINE

ON THE UPTAKE OF NOREPINEPHRINE

The influence of nicotine on the uptake of norepinephrine can be conveniently studied by administering the nicotine prior to the intravenous injection of tritiated norepinephrine as described above. Similar experiments can be carried out on the perfused guinea pig heart where, in addition to net uptake, the arterial-venous difference can also be studied. This can be accomplished by measuring the perfusate content as well as the tissue content of norepinephrine.

10035467297

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

EXTRACTION, SEPARATION AND ANALYSIS OF CATECHOLAMINES AND METABOLITES

Animals injected, sacrificed (decapitation), tissues dissected out and weighed.

↓

Homogenization (0.4 N perchloric acid, Ultra-turrax apparatus)

↓

Centrifugation (10,000 x g - 10 mm)

↓

Ascorbic acid, EDTA added
pH adjusted to 8.3-8.5 (NaOH)

→ Aliquot (taken for total radioactivity determination)

↓

Alumina Columns
washed with water

→ Catecholamines eluted with 0.2 N HCl

↓

Effluent + washings
(o-methylated metabolites)

↓

Adjusted to Ph 6.5

↓

Dowex column (50W-X4)
washed with water
eluted with 6 N HCl+Ethanol

↓

Assayed for Normetanephrine in Scintillation Counter

Catecholamines eluted with 0.2 N HCl

↓

Aliquot (assay of norepinephrine)

↓

Assayed in Scintillation Counter

Catecholamines eluted with 0.2 N HCl

↓

Aliquot (deaminated metabolites)

↓

6 N HCl, NaCl added

↓

Ethylacetate

↓

Contrifugation

↓

Assayed in Scintillation Counter

1003546798

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REFERENCES

Burack, W. R. and P. R. Draskoczy. The turnover of endogenously labelled catecholamines in several regions of the sympathetic nervous system. J. Pharmacol. exp. Ther., 144: 66, 1964.

Carlsson, A. Pharmacological depletion of catecholamine stores. Pharmacol. Rev., 18: 541, 1966.

Costa, E., Boullin, D. J., Hammer, W., Vogel, W. and B. B. Brodie. Interactions of drugs with adrenergic neurons. Pharmacol. Rev., 18: 577, 1966.

Daly, J. W., Creveling, C. R. and B. Witkop. The chemorelease of norepinephrine from mouse hearts. Structure-activity relationships. I. Sympathomimetic amines and related amines. J. Med. Chem., 9: 273, 1966.

Daly, J. W., Creveling, C. R. and B. Witkop. The chemorelease of norepinephrine from mouse hearts. Structure-activity relationships. II. Drugs affecting the sympathetic and central nervous system. J. Med. Chem., 9: 280, 1966.

Glowinski, J. and R. J. Baldessarini. Metabolism of norepinephrine in the central nervous system. Pharmacol. Rev., 18: 1201, 1966.

Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain. I. The disposition of (H^3) norepinephrine, (H^3) dopamine and (H^3) dopa in various regions of the brain. J. Neurochem., 13: 655, 1966.

Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain. III. Subcellular distribution of endogenous and exogenous catecholamines in various brain regions. Biochem. Pharmacol., 15: 977, 1966.

Hertting, G., Axelrod, J. and R. W. Patrick. Actions of cocaine and tyramine on the uptake and release of H^3-norepinephrine in the heart. Biochem. Pharmacol., 8: 246, 1961.

Hornykiewicz, O. Dopamine (3-hydroxy tryptamine) and brain functions. Pharmacol. Rev., 18: 925, 1966.

Iversen, L. L. and J. Glowinski, O. Regional studies of catecholamines in the rat brain. II. Rate of turnover of catecholamines in various brain regions. J. Neurochem., 13: 671, 1966.

Kopin, I. J., Axelrod, J. and E. Gordon. The metabolic fate of H^3-epinephrine and C^{14}-metanephrine in the rat. J. Biol. Chem., 236: 2109, 1961.

Montanari, R., Costa, E., Beaven, M. A. and B. B. Brodie. Turnover rates of norepinephrine in hearts of intact mice, rats and guinea pigs using tritiated norepinephrine. Life Sci., 2: 232, 1963.

10035467 99

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Nash, C. W., Wolff, S. A. and B. A. Ferguson. The action of sympathomimetic amines on the release of noradrenaline from isolated perfused rat hearts. Fed. Proc., 26: 570, 1967.

Neff, N. H., Tozer, T. N., Hammer, W. and B. B. Brodie. Kinetics of release of norepinephrine by tyramine. Life Sci., 4: 1869, 1965.

Noble, E. P., Wurtmann, R. J. and J. Axelrod. A simple and rapid method for injecting H^3-norepinephrine into the lateral ventricle of the rat brain. Life Sci., 6: 281, 1967.

Trendelenburg, U. Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. Pharmacol. Rev., 18: 629, 1966.

Westfall, T. C. Effect of nicotine and nicotine analogues on tissue and urinary catecholamines in the rat. Acta physiol. Scand., 63: 77, 1965.

Westfall, T. C. Tobacco alkaloids and the release of catecholamines, in tobacco alkaloids and related compounds. Ed. by U. S. von Euler, Pergamon Press, 4: 179, 1965.

Westfall, T. C., Fleming, R. M., Fudger, M. K. and W. G. Clark. Effect of nicotine and related substances on amine levels in the brain. Ann. N. Y. Acad. Sci., 142: 83, 1967.

Zeman, W. and J. R. M. Innes. Craigie's Neuroanatomy of the Rat. Academic Press, New York, 1963.

1003546800

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

FUNDS FOR THE PURCHASE OF A LIQUID SCINTILLATION SPECTROMETER

Present Request	$ 7,500.00
Additional Funds:	
Second year budget of a grant entitled, Action of Nicotine on Subcellular Distribution of Catecholamines in Brain and Heart, Council for Tobacco Research - U.S.A.	3,000.00
Research and Development Fund University of Virginia School of Medicine	5,000.00
Total. .	$15,500.00
Cost of Instrument. .	$15,500.00

1003546801

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

9. Facilities and Staff Available

A well equipped Department of Pharmacology with adequate laboratory and office space is available. Equipment available for this project includes: Farrand Model A photoelectric fluorometer with the necessary filter combinations for differential estimation of epinephrine and norepinephrine, the use of (on loan from Cancer Research Laboratory, located on floor below) an Aminco-Bowman spectrophotofluorometer, Beckman expandomatic pH meter, chromatographic columns for alumina absorption, ultra-turrax high speed tissue homogenizer, International refrigerated centrifuge, Gilson Medical Electronics fractionator, Beckman spectrophotometers, analytical balances, Grass polygraphs, and Type 40 rotor for ultracentrifuge. The use of a Spinco preparative ultracentrifuge located in the Department of Biochemistry on the floor above is also available. Our Department has a well equipped shop and dark room as well as rooms for animals within the Department and access to the general animal quarters from the School of Medicine.

Staff.

1. Thomas C. Westfall, Ph.D.
   Principle Investigator

2. Hirofumi Osada, M.D.
   Research Fellow

3. Leslie Frank
   Research Assistant

4. William Moore
   Graduate Student

10035468O2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Name: Thomas C. Westfall

Date of Birth: R

Place of Birth: Latrobe, Pennsylvania

Marital Status: ___ R

Education:

1959 A.B., Biology and Chemistry, West Virginia University
1961 M.S., Pharmacology, West Virginia University
1962 Ph.D., Pharmacology, Dr. Daniel T. Watts, supervisor, West Virginia University

Educational Awards:

1959-1962. National Institutes of Health Predoctoral Traineeship in Pharmacology.
1963-1964. National Heart Institute Postdoctoral Award.

Academic Positions Held:

1962-1963. Instructor in Pharmacology, West Virginia University Medical Center.
1963-1964. Research Fellow of National Heart Institute, Department of Physiology, Karolinska Institute, Stockholm, Sweden, Professor U. S. von Euler, supervisor.
1964-1965. Assistant Professor of Pharmacology, West Virginia University Medical Center.
1965- Assistant Professor of Pharmacology, University of Virginia School of Medicine.

Teaching Experience:

1962 thru 1965. Gave approximately 20 lectures per year to medical, nursing and graduate students at West Virginia University Medical Center. This involved participation in the laboratory course of instruction to the same groups.

1966 to present. Gave about one-fourth of the lectures in Pharmacology to the sophomore medical class at the University of Virginia. This also involved active participation in all laboratory exercises. Active teaching at the graduate level and supervision of graduate students was also carried out.

Committee Assignments (University of Virginia School of Medicine):

1965-1966. Research Society, School of Medicine.
1966-1967. Search Committee for Pharmacology Chairman.

10035468O3

XEROX COPY XEROX XEROX XEROX

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

1966-1967. Search Committee for Psychiatry Chairman.
1967- . AD HOC Curriculum Committee of Basic Sciences.
1967- . AD HOC Committee, Cell Biology course for first year medical students.

Society Memberships:

REDACTED REDACTED REDACTED

Professional Lectures (by invitation):

University of Texas School of Medicine, Galveston, Texas.
Turku University School of Medicine, Turku, Finland.
Karolinska Institute, Stockholm, Sweden.
Philadelphia General Hospital, Cardiology Division, Philadelphia, Pa.
Duke University School of Medicine, Durham, N. C.
Medical College of Virginia, Richmond, Va.

Symposia Participation (by invitation):

Fourth International Wenner-Gren Center Symposium. Tobacco Alkaloids and Related Compounds. Held in Stockholm, Sweden, February, 1964. Paper presented: Tobacco Alkaloids and the Release of Catecholamines.

New York Academy of Sciences Symposium on The Effects of Nicotine and Smoking on the Central Nervous System. Held in New York City, April 7-9, 1966. Paper presented: Effect of Nicotine and Related Substances on Amine Levels in the Brain.

American Medical Association Workshop on Tobacco and Health. Held at Broadmoor Hotel, Colorado Springs, Colorado, November 1-3, 1966. Paper presented: Influence of Nicotine on Catecholamine Metabolism.

Major Fields of Investigation:

Central and peripheral autonomic effects of nicotine.
Influence of drugs on uptake, storage, release and inactivation of biogenic amines.

10035946804

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIBLIOGRAPHY

of

Thomas C. Westfall

I. Major Publications

1. Westfall, T. C. and Watts, D. T. The effect of reserpine on the cardiovascular response to smoking. J. Appl. Physiol., 17: 471, 1962.

2. Westfall, T. C. and Watts, D. T. The effect of cigarette smoke on epinephrine secretion in the dog. Proc. Soc. Exp. Biol. Med., 112: 843, 1963.

3. Westfall, T. C. and Watts, D. T. Catecholamine excretion in smokers and nonsmokers. J. Appl. Physiol., 19: 40, 1964.

4. Westfall, T. C. and Watts, D. T. The effect of nicotine on brain and urinary amines in the rat. J. Neurochem., 11: 397, 1964.

5. Westfall, T. C. Tobacco alkaloids and the release of catecholamines in Tobacco Alkaloids and Related Compounds, Ed. by U. S. von Euler, Pergamon Press, 4: 179, 1965.

6. Westfall, T. C. Effect of nicotine and nicotine analogues on tissue and urinary catecholamines in the rat. Acta physiol. Scand., 63: 77, 1965.

7. Westfall, T. C. Uptake and exchange of catecholamines in rat tissues after d and l-adrenaline. Acta physiol. Scand., 63: 336, 1965.

8. Westfall, T. C. and Peach, M. J. Action of angiotensin on myocardial and renal catecholamines in the rabbit. Biochem. Pharmacol., 14: 1916, 1965.

9. Westfall, T. C., Cippoloni, B. and Edmundowicz, A. Influence of propranolol on the hemodynamic changes and plasma catecholamine levels following cigarette smoking and nicotine. Proc. Soc. Exp. Biol. Med., 123: 174, 1966.

10. Westfall, T. C., Fleming, R. M., Fudger, M. K. and Clark, W. G. Effect of nicotine and related substances on amine levels in the brain. Ann. N. Y. Acad. Sci., 142: 83, 1967.

1003546805

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Westfall, T. C. Accumulation of norepinephrine in rat tissue following treatment with three beta adrenergic antagonists. Arch. int. Pharmacodym., 167: 69, 1967.

12. Westfall, T. C. and Anderson, G. P. Influence of nicotine on catecholamine metabolism in the rat. Arch. int. Pharmacodyn., (in press) 1967.

13. Westfall, T. C. Effect of beta adrenergic blockers on the noradrenaline content of rat heart and spleen before and after noradrenaline infusion. Brit. J. Pharmac. Chemother., (in press) 1967.

In Preparation

Influence of Beta adrenergic antagonists on norepinephrine storage in the perfused guinea pig heart.

Action of nicotine on subcellular amine levels in brain and heart.

Influence of adrenalectomy on repletion of catecholamine stores following depletion with various pharmacological agents.

II. Abstracts (* delivered paper)

1. Westfall, T. C.* and Watts, D. T. The effect of reserpine on the cardiovascular response to smoking. Fed. Proc., 20: 89, 1961.

2. Westfall, T. C.* and Watts, D. T. The effect of cigarette smoke on epinephrine secretion in the dog. Fed. Proc., 21: 193, 1962.

3. Westfall, T. C.* and Watts, D. T. Catecholamine excretion in smokers and nonsmokers. Fed. Proc., 22: 1509, 1963.

4. Peach, M. J.* and Westfall, T. C. Action of angiotensin on myocardial catecholamines in the rabbit. Fed. Proc., 24: 488, 1965.

5. Westfall, T. C.* Influence of pronethalol, propranolol and iproveratril on uptake and storage of norepinephrine. Fed. Proc., 25: 260, 1966.

6. Westfall, T. C.*, Fleming, R. M., Fudger, M. K. and Clark, W. G. Effect of nicotine and related substances on amine levels in the brain. Symposium abstract. Symposium on The Effects of Nicotine and Smoking on the Central Nervous System. N. Y. Acad. Sci., April, 1966.

7. Westfall, T. C.* Uptake and storage of norepinephrine following the administration of three beta adrenergic antagonists. Va. J. Sci., 17: 354, 1966.

1003546806

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Westfall, T. C.* Influence of nicotine on catecholamine metabolism. Symposium abstract. Tobacco and Health. Amer. Med. Assn., November, 1966.

9. Westfall, T. C.* Influence of beta adrenergic antagonists on the norepinephrine content in rat heart before and after NE infusion. Fed. Proc., 26: 569, 1967.

11. Westfall, T. C.* Influence of beta adrenergic blockers on norepinephrine storage in the perfused guinea pig heart. Va. J. Sci., 00: 000, 1967.

12. Westfall, T. C.* The effect of beta adrenergic blocking drugs on the norepinephrine level in the perfused guinea pig heart following NE infusion. The Pharmacologist, 9: 249, 1967.

III. Texts

Contributor to:

Medicinal Chemistry, Alfred Burger, Editor, third edition, John Wiley and Sons, Inc., Interscience Publishers, New York.

Introduction to Neuropharmacology (co-author, with E. D. Brand).

10035468O7

XEROX COPY XEROX COPY XEROX COPY XEROX COPY

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Name: Hirofumi Osada

R R

Place of Birth: Kawasaki, Japan

R R

Education:

Yokohoma Municipal University, M.D., March 1959.
University of Tokyo Hospital, Internship, April 1959 - March 1960.
University of Tokyo, Doctorate of Medical Science (Internal Medicine), March 1964.

Academic Positions:

1964-1966. Instructor in Internal Medicine, University of Tokyo.
1966-1967. Research Fellow, Division of Cardiology, Philadelphia General Hospital, Philadelphia, Pa.
1967- . Research Fellow, Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Va.

Society Memberships

REDACTED REDACTED

10035468O8

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIBLIOGRAPHY

of

Hirofumi Osada

Publications

1. Kobayashi, T., Ito, Y., Kobayashi, M., Kajihara, T., Takeuchi, M., Shimizu, K. and Osada, H. Clinical experience and effects on serotonin and catecholamine metabolism of new anti-anginal drug (Nialamide). In Japanese. J. Therap., 43: 1961, 1961.

2. Yoshimura, S., Kobayashi, T. and Osada, H. Acute cardiac death. In Japanese. Diagnosis and Therapy, 50: 925, 1962.

3. Kobayashi, T., Ito, Y., Kajihara, T., Kishii, T., Osada, H., Komizo, U., Sugai, M., Yoshimura, S., Kono, R., Makai, N. and Aoki, T. Acute cardiac death of unknown etiology. In English. Jap. Heart J., 3: 442, 1962.

4. Kobayashi, T., Ito, Y., Kajihara, T., Shimizu, K., Kishii, T., Osada, H. and Komizo, U. Clinical experience and effects of α-methyl dopa on serotonin and catecholamine. In Japanese. J. Therap., 44: 1888, 1962.

5. Kobayashi, T., Ito, Y., Kajihara, T., Shimizu, K., Kishii, T., Osada, H. and Komizo, U. A new anti-hypertensive drug (α-methyl dopa). In Japanese. J. Geriatrics, 7: 330, 1963.

6. Ejima, M., Osada, H. and Ikeda, T. Paraganglioma. In Japanese. Endocrinology and Metabolism, 4: 99, 1963.

7. Yoshimura, S., Kobayashi, T. and Osada, H. Acute cardiac death. In Japanese. J. Jap. Med. Assn., 49: 1015, 1963.

8. Kobayashi, T., Osada, H. and Komizo, U. α-methyl dopa as a anti-hypertensive drug. In Japanese. Respiration and Circulation, 11: 507, 1963.

9. Osada, H., Komizo, U., Kondo, K., Kishii, T., Kajihara, T., Kobayashi, T. and Okano, S. Experimental and clinical trial of H^3-catecholamine, especially as to α-methyl dopa. In Japanese. Saishin-Igaku, 19: 198, 1964.

10. Kobayashi, T., Kajihara, T. and Osada, H. Some considerations on the causative diseases of acute cardiac death. In Japanese. J. Chest. Dis., 8: 465, 1964.

11. Yoshimura, S., Kobayashi, T., Kajihara, T. and Osada, H. Acute cardiac death of unknown etiology. In Japanese. J. Chest. Dis., 8: 529, 1964.

1003546809

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

12. Osada, H. The effects of reserpine, α-methyl dopa, nialamide and dexamethazone on the uptake and metabolism of H^3-catecholamine. In English. Jap. Heart J., 5: 224, 1964.

13. Kobayashi, T., Kajihara, T. and Osada, H. Progress on research in the world in 1964. Etiology, diagnosis and therapy of hypertension. In Japanese. Jap. Med. J., 2129: 3, 1965.

14. Kobayashi, T. and Osada, H. Anti-hypertensive effects of reserpine. In Japanese. Jap. Med. J., 2147: 139, 1965.

15. Kobayashi, T. and Osada, H. Basal and casual blood pressure. In Japanese. Jap. Med. J., 2140: 130, 1965.

16. Kobayashi, T., Osada, H., Kondo, K. and Tawara, I. Anti-anginal and anti-arrhythmic effects of propranolol. In Japanese. J. Therap., 48: 775, 1966.

17. Kobayashi, T., Kajihara, T., Osada, H., Komizo, U. and Ishii, K. The effect of nitroglycerine on H-catecholamine metabolism. In English. Jap. Heart J., 7: 430, 1966.

18. Kobayashi, T., Kajihara, T., Osada, H. and Okada, T. The effects of ethacrynic acid. In Japanese. J. Therap., 1966 (under contribution).

19. Osada, H. Review and Practice. The methods of measurements of catecholamine in urine, blood and tissues. In Japanese. Library for Chemical Analysis, Vol. 6, Jap. Chemical Analystics Assn, in Tokyo (under contribution).

10035468 10

XERO COPY XERO XERO

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

MODIFIED

100354811

Source: https://www.industrydocuments.ucsf.edu/docs/pnmh0000

#613-M CLARK

1003546812

Source: https://www.industrydocuments.ucsf.edu/docs/ftcm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. September 5, 1967

MEMORANDUM

TO: The committee comprising Dr. Little, Chm., Dr. Loosli and Dr. Cattell.

FROM: Robert C. Hockett

SUBJECT: A modified proposal from R. Ernest Clark, Ph.D. and Donald B. Giddon, D.M.D., Ph.D. - No. 613-M.

We enclose herewith a modified proposal from Drs. R. Ernest Clark and Donald B. Giddon of Tufts University School of Dental Medicine, Boston, Massachusetts.

Their original proposal, #613, considered by the Board in May, 1967, did not receive approval. Four questions in particular were raised in the discussion. These questions were relayed with Dr. Clark by the staff and have been taken into consideration in the revised proposal which he now asks the Board to review.

In order to maintain the continuity of the proposal a copy of the original proposal is enclosed along with a copy of the original memo.

The budget appears to be reasonable in light of the fact that the principal investigator intends to devote a good deal of time to this proposed investigation. You will note that we are not competing in the budget for graduate students, and possibly questionable quality that may result from the use of departmental graduate students. They are asking for one year's support only and intend to submit a new proposal based on the results and data obtained from this exploratory effort if it looks promising.

Vincent F. Lisanti
Robert C. Hockett

1003546813

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC

NO. 613-M

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Little, Chm.
Dr. Loosli
Dr. Cattell

Application For Renewal of Research Grant

First ☒ Second ☐

Date: August 21, 1967

1. Name of Investigator(s): (include title and degrees)

R. Ernest Clark, Ph.D. Associate Professor of Psychology (effective 9/1/67)
Donald B. Giddon, D.M.D. Ph.D. Professor and Chairman, Dept. of Social Dentistry

2. Institution & Address: Tufts University School of Dental Medicine
136 Harrison Avenue
Boston, Massachusetts

3. Short Title of Project: A Psychophysiological Study of the Act of Reaching for a Cigarette.

4. Proposed Renewal Starting Date: (Anniversary or other)

September, 1967.

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

Re recommendations of the Council:

1. It has been the intention of the investigators to consider the "factors as potential modifiers of habits" indicated (i.e., financial security, etc), but more comprehensibly within the framework of the reported findings of Matarazzo, and Saslow (Psychological and related characteristics of smokers and nonsmokers, Psychol. Bull, 1960, 57, 493-513, Table 6 - Differentiating Characteristics).

2. Because glucose taken orally can block the usual cardiac effects of smoking a cigarette, and because of the high positive correlation between smoking and coffee drinking (usually with sugar), it remains reasonable to determine the smokers joint usage of cigarettes and coffee (especially with sugar) to "regulate" his bodily processes.

3. It is certainly of future interest to evaluate psychophysiological differences among smokers associated with differences in background characteristics as to why they started to smoke and why they continued, but such questions are beyond the immediate scope of this project.

4. A small group of nonsmokers is necessary to evaluate normal (non-smoker related) variation in psychophysiological measurement during the 2 hr experimental sessions. It is certainly true, however, that the investigators should attempt to select non-smokers to be homogenious as to their reasons for being non-smokers.

6. Give a Brief Statement of your Working Hypothesis if altered or modified:

Same as previous application.

1003546814

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

The original proposal indicated that questionnaires would be given to about 600 students in the principal investigator's introductory psychology classes at Northeastern University. This can no longer be done. These classes are now within a week of completion. Should a grant be received for the proposed research, other subjects will have to be sought. As many as possible will be tested, but the actual number and sources are unknown. When potential subject samples (students in various colleges and universities in the Boston region) have been identified, questionnaires will be administered to obtain information regarding use of tobacco products, coffee, alcohol, sugar, as well as information to differentiate among the smoker and nonsmoker groups. Consistent with the recommendations of the Tobacco Council, the two groups will be selected to maximize intra-groups homogeneity.

8. Additional Requirements: None.

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

None.

10. Publications or Papers in Press resulting from the Project or closely related work

Same as previous application.

1003546815

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Budget (for coming year)

A. Salaries (Personnel by names or category)	% time	Amount
Professional		
R.E. Clark, Ph.D.	40	7000
D.B. Giddon, D.M.D., Ph.D.	as needed	--
Technical		
Research Assistant	100	5200
Secretary	25	1200
Salary corollaries		1612
Sub-Total		15,012
B. Consumable Supplies (list by categories)		
Polygraph Supplies (paper, etc.)		
Thermistors, transducers		750
Sub-Total		
C. Other Expenses (itemize)		
Electronic maintenance & consultation	$900	
Travel (Psychophysiological meeting, San Diego)	$500	
Publications and fees	$200	
Payment to Subjects	$750	2350
Sub-Total		
D. Permanent Equipment (itemize)		
1 Beckman 2-Channel offner polygraph and accessories		2500
E. Overhead (15% of A+ B +C)		2716.80
Total		23,328.80

New proposal will be submitted at the end of the first year according to the findings of the exploratory study.

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature R.E. Clark
Director of Project
617-[illegible]
Telephone

Signature H.A. Davis
Business Officer of the Institution
Telephone

10035468 16

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Same as previous application

	Title of Project	Source	Amount	Duration
Current				
Pending				

10035468l7

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

September 18, 1967

MEMORANDUM

TO: The committee comprising Dr. Lynch, Chm., Dr. Reimann and Dr. Loosli.

FROM: Robert C. Hockett

SUBJECT: Request for grant supplement from John E. Craighead, M.D. of Harvard Medical School - No. 550-S.

We enclose herewith a request from Dr. John E. Craighead of Harvard's Department of Pathology for a supplement to his current year's grant in the amount of $2,572. Reasons for the request are stated briefly in his letter.

This request reached us after mailing of the meeting book for September 23-24. If the committee feels that the matter can nevertheless be settled at the forthcoming meeting, it can be added to the agenda. The project is a young one, but a progress report dated January 30, 1967 gave some account of the work going on and this appears to be both promising and quite relevant to the main-stream interests of the Council.

R. C. H.

1003546818

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

VIRUS, Previous Infection

#550-S
Activated: 1/1/67

COMMITTEE:
Dr. Lynch, Chm.
Dr. Reimann
Dr. Loosli

ARTHUR T. HERTIG, M.D.
SHATTUCK PROFESSOR
OF PATHOLOGICAL ANATOMY

DEPARTMENT OF PATHOLOGY
HARVARD MEDICAL SCHOOL
25 SHATTUCK STREET
BOSTON, MASS. 02115

September 6, 1967

Mr. W. T. Hoyt, Executive Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue,
New York, N. Y. 10017

Dear Mr. Hoyt:

I am writing pursuant to my telephone conversation with your staff member regarding a revision of the budget for the grant entitled "Biology and Cytopathic Effects of Respiratory and Oncogenic Viruses in Organ Cultures of Human Respiratory Tract Tissue." As indicated in my report of June 30, 1967, the work supported by this grant has progressed most satisfactorily although costs have been greater than initially anticipated. This is in part due to the success of our initial studies but more because increases in supply costs and salaries have been substantial during 1967. In addition we have found it necessary to utilize commercially supplied cell cultures in order to conduct certain of our investigations. I believe a modest increase in our budget will make it possible to conduct our studies productively and with a high degree of efficiency.

Enclosed please find a revised budget for the 3 years of grant support. You will note that the budget for the present first year has been increased by $2,572. This will compensate for a wage adjustment for non-professional personnel made by the University on July 1, 1967, increased demands on our part-time histologist, and the use of commercially supplied primary human cell cultures. Proportional increases have been made in the revised budget for the second and third year. These changes anticipate the growing scope of our work and increased costs. I trust the Progress Report submitted to your office on January 30, 1967 will provide detailed information on our work. Should a more comprehensive up-to-date evaluation of these studies be required, please feel free to contact me. I would be happy to provide specific details on matters of salaries and supply costs.

Sincerely yours,

John E. Craighead

John E. Craighead, M.D.
Assistant Professor

JEC:nsc
Enc.

1003546819

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REVISED BUDGET

Grant: Biology and Cytopathic Effects of Respiratory and Oncogenic Viruses in Organ Cultures of Human Respiratory Tract Tissue

Principal Investigator: J. E. Craighead

	Year 1	Year 2	Year 3
Principal Investigator	2790	2930	3077
Retirement, Social Security, etc. 16%	446	469	492
Technician (full time)	5175	5800	6000
Histologist (shared)	1500	2000	2100
Secretary, bookkeeper, dishwasher, (shared) 9 1/4%	1600 765	1800 888	1900 925
Supplies			
Media, serum, chemical	1200	1250	1300
Commercial-supplied cell cultures	1500	2000	2200
Glassware	500	500	500
Disposable plastics	500	550	600
Histology supplies	200	200	200
Paper, secretarial supplies	100	100	100
Miscellaneous	300	300	300
Equipment			
Replacement parts and service	150	150	150
Travel	150	150	150
	16,876	19,087	19,994
Indirect Costs 15%	2,531	2,863	3,199
Total	19,407	21,950	23,193

1003546820

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

NEW APPLICATIONS FROM
PRESENT GRANTEES

100354821

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#338-A HOMBURGER

1003546822

Source: https://www.industrydocuments.ucsf.edu/docs/pfdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

September 7, 1967

Deferred for investigation

MEMORANDUM

TO: The committee comprising Dr. Sommers, Chm., Dr. Jacobson, Dr. Loosli and Dr. Reimann.

FROM: Robert C. Hockett

SUBJECT: New research proposal from Freddy Homburger - No. 338-A.

We enclose herewith a new research proposal from Freddy Homburger of the Bio-Research Institute, Inc., of Cambridge, Massachusetts, which is intended to succeed the present - #338-R4.

As he points out in his application, CTR Grant - #338 - was activated on October 1, 1962 as a five-year plan which will terminate on October 1, 1967. This was the so-called "basic research" grant which has been considered quite separate from the concurrent grant - #456 for smoke inhalation studies. The latter is considered by Homburger to be in the nature of a contract. He now submits a summary review of progress during these five years, which is enclosed with a manuscript:

"Experimental Studies on the Inhibition of Carcinogenesis by Cigarette Smoke Condensates and Carcinogen - Related Substances". F. Homburger, A. Treger and E. Boyer. To be published in the Journal of the National Cancer Institute.

The present application proposes a group of studies to follow-up and build upon the findings of project - #338 with practical objectives in view. Because of its relation to - #338, we have designated it as No. 338-A to maintain continuity of labelling yet recall that it is a new proposal insofar as commitments are concerned.

This proposal was generated without any instigation from the staff and is transmitted for consideration on its merits.

R. C. H.

P.S. The manuscript mentioned in the enclosed progress report - "Acceleration of Growth of Chemically Induced Tumors by Use of Transplantation Technic", F. Homburger and A. Treger, Cancer Res. 27: 1205-1213, 1967)., is forthcoming and will be forwarded when received.

R.C.H.

1003546823

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

NO. 338-A

Cf. #338

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

Activated on 10/1/62 and renewed annually thru October 1, 1966. Preceded by #29B, #212 & #224 - 1955 - 1962 incl. #456 is concurrent on a five year plan.

COMMITTEE:
Dr. Sommers, Chm.
Dr. Jacobson
Dr. Loosli
Dr. Reimann

Application For Research Grant

Date: August 18th, 1967

1. Name of Investigator: Freddy Homburger, M.D.

2. Title: President and Director

3. Institution & Address: Bio-Research Institute, Inc.
9 Commercial Avenue
Cambridge, Massachusetts 02141

4. Project or Subject: BIOASSAY OF TOBACCO SMOKE CONDENSATES AND RELATED PROBLEMS

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

INTRODUCTION

It is realized that our current grant from CTR has been made for one year without further commitment in order to enable us to conclude a five-year program initiated late in 1962.

The present application is to request the Council to consider support for another five-year program, based upon our past performance summarized in the progress report.

The three major projects for which we seek long-term support are the following:

1. Acceleration of growth of chemically induced tumors for the purpose of developing rapid carcinogen testing methods.

2. Systematic study of inhibitors of chemical carcinogenesis with the aim to neutralize alleged carcinogens contained in cigarette smoke condensates.

100354824

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

3. Skin-painting studies in mice to measure under comparable conditions the decline of carcinogenicity and of co-carcinogenicity for mouse skin that appears to have occurred since 1960 in cigarette smoke condensate.

Each of these projects is based on many years of experience in the particular field and clearly promises to yield valuable fundamental scientific knowledge and will contribute to the technology necessary to formulate cigarettes that will produce smoke condensates incapable of producing cancers when painted on the skin of mice.

1) Acceleration of Carcinogen Testing

There are efforts underway in many laboratories to develop rapid screening procedures for the detection of carcinogenic substances. These range from in vitro tissue culture work to the use of neonatal mice, newts and other species. The destruction of sebaceous glands and the increased lethality of U.V. light for paramecia and other biological phenomena have been correlated with carcinogenic potency. However, the most reliable carcinogenesis test would still be the production of tumors in a mammalian species in a sufficiently short time to make it practical to detect even weak cancer-causing chemicals.

Our studies on the transfer of multiple pooled carcinogen injection sites represent the first significant step in this direction. We believe that it will be possible to obtain even shorter times of latency than so far possible by extracting from the initial carcinogen-injection or -application sites large numbers of transformed cells for transfer into fresh hosts.

Initial experiments on this subject are already underway. The subcutaneous injection sites of C57BL/6 mice are excised after 3 to 5 weeks of contact with carcinogen (benzo[rst]pentaphene is being used as standard carcinogen) and after mechanical dispersion by means of Snell's cytosieve, the cell suspensions are centrifugated in Ficoll®, a neutral high molecular dextran-like polysaccharide of low osmotic pressure. The cells are thereby separated according to their specific gravity and the various cell layers, some of which will contain concentrated amounts of malignant cells, are injected into fresh hosts. By this method, it is possible to inject into a single mouse many times the number of transformed cells coming from numerous induction sites. Based on the studies of several authors using transplanted tumors and confirmed by our own work, the larger the number of transferred malignant cells, the more rapid the growth of tumors. It is believed that it may be possible to get a 100% tumor yield 2 weeks after transfer into new hosts or 5 to 7 weeks after the carcinogen was first injected.

1003546825

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Bioassay of Tobacco Smoke Condensates and Related Problems

In the mouse, the first cytologically malignant cells are found in carcinogen injection sites in the subcutaneous tissue five weeks after injection of carcinogen. By transfer of pooled multiple injection sites, we were observing changes in from 2 to 3 weeks after carcinogen injection but not earlier. Thus, the shortest theoretically possible test period would be 4 weeks.

In hamsters, on the other hand, Nettleship and Smith (Proc. Soc. Exp. Biol. Med. 74:800-802, 1950) showed morphologically transformed fibroblasts 24 hours after injection of methylcholanthrene. Hence, it would appear that the subcutaneous tissue of hamsters would lend itself even better than that of the mouse to carcinogen testing. The times of latency in hamsters for subcutaneously induced tumors is the same as in mice. It is likely (assuming that Nettleship's observations can be confirmed) that transfer of multiple injection sites in hamsters and even more so, transfer of transformed cell concentrates, will produce palpable tumors in a shorter period than in mice. Such transfers could be made 1 or 2 days after injection of carcinogen and the shortest possible test period would be 2 to 3 weeks.

In addition, chromosome studies are readily feasible in hamsters and tumor cells transferred from females into males could be identified as belonging to the original female host and hence as induced by the carcinogen injected into the first (female) host.

We propose to establish during the next 2 to 3 years a tissue culture laboratory under the direction (either part time or later on full time) of Dr. Janis Gabliks, currently Associate Professor of Cell Biology at Massachusetts Institute of Technology, and to carry out the above described studies.

In addition, it will then become possible to extend the work of Berwald and Sachs on transformation of hamster fibroblasts in vitro and to transfer large numbers of cells exposed to carcinogens in vitro back into hamster cheek pouches. In this way, it may be possible to obtain tumor growth even more rapidly than is possible with in vivo systems alone.

We are quite confident that these techniques applied to subcutaneous tissue, epidermis and lung tissue of mice and hamsters will make such procedures as mouse-skin painting obsolete and replace them by carcinogen tests lasting less than two months and having as endpoints histologically demonstrable malignant tumors, the truly neoplastic nature of which can be ascertained by serial transplantation.

1003546826

While these studies will initially be done with chemical carcinogens (strong, weak and intermediate), we shall soon be able to apply them as

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

well to tobacco smoke condensates, since the condensate producing machine of Bio-Research Consultants, originally scheduled for this spring, will be in operation later this summer and condensate will be available in adequate amounts.

2) Systematic Study of Inhibitors of Chemical Carcinogenesis

While Falk and Kotin showed that reduction derivatives of polycyclic hydrocarbons inhibit the carcinogenic effect of these hydrocarbons, we found that oxidative derivatives also have this effect.

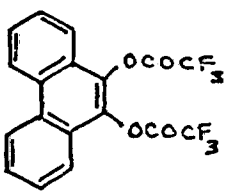
The quinones appear to be a group of special interest because they possess chemotherapeutic (in contrast to merely carcinogenesis inhibiting) effects. The hypothesis that hydrogen peroxide, which may form from quinones, is the cytotoxic agent is susceptible to test by synthetizing the aza derivatives corresponding to the quinones which, if the peroxide hypothesis were correct, would not possess the chemotherapeutic activity of the quinones. Conversely, the di-quinone should be more active.

It is the purpose of a systematic study of derivatives of benzo [rst]pentaphene, of other polycyclic hydrocarbons and of terpenes (such as limonene) and their derivatives to find those compounds that are most active in counteracting the carcinogenic effects of polycyclic hydrocarbons and that are themselves least carcinogenic. Such compounds could be used eventually to neutralize the carcinogenic effects (as tested by our own new rapid methods and by skin painting) of cigarette smoke condensates.

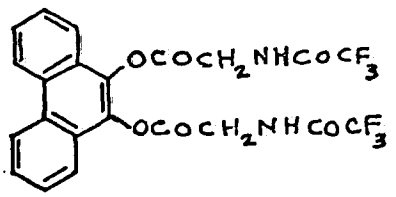
In order to render these compounds more volatile and better suited for use as adjuvants in the tobacco blends, it is suggested to prepare and test the trifluoroacetyl derivatives of some hydroquinones and their glycyl esters, which are known to be precursors of the cytotoxic quinones in vivo.

These compounds are represented by the following examples:

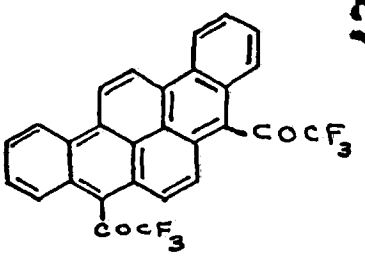
1. 9,10-Phenanthrene hydroquinone di(trifluoroacetate), IV
2. 9,10-Phenanthrene hydroquinone-bis-trifluoroacetylglycyl ester, V
3. 3,4,9,10-dibenzpyrene-5,8-di(trifluoroacetoxy), VI



IV



V



VI

100354827

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Bioassay of Tobacco Smoke Condensates and Related Problems

This approach is based on methods used in gas chromatography for the volatilization of amino acids (see, for example, Cruickshank and Sheehan, Anal. Chem. 36:1191, 1964). The trifluoroacetate group is known to split very easily, and the trifluoroacetic acid and its derivatives are completely non-toxic and physiologically inert (see "Toxic Aliphatic Fluorine Compounds" by F. L. M. Pattison, Elsevier Pub. Co., 1959, pp. 20;27;62).

3) Skin-Painting Studies in Mice

Skin painting in mice has been used since Croninger, Graham and Wynder's early work, for lack of a better method, as a measure of carcinogenicity of smoke condensates.

There are indications that present day cigarettes may be less carcinogenic and less co-carcinogenic in terms of mouse skin response than those originally studied by Wynder, Kensler and ourselves. Such observations on reduced carcinogenicity of cigarette smoke condensates have been published by Wynder and by Bock. However, neither of these authors was in a position to prepare at the time of his latest study condensates from cigarettes used in his earlier (more carcinogenic) experiments. We have some 20,000 cigarettes made in 1960 of cigar tobacco which have been preserved and which could serve as a control for a comparison between mouse carcinogenicity of condensates of 1960 and of 1967.

The evidence which suggests such an experiment is shown in Table I, summarizing our own mouse-skin painting experiments using condensates from various unfiltered cigarettes done in 1960, 1963, 64, 65 and 66.

There is a strong suggestion here that the mouse-skin carcinogenicity has declined and, even more striking, that co-carcinogenicity has practically disappeared. While the cigarettes used were the same brand in most of these studies, the source of the condensates, the machines used for smoking and the handling of the condensates were different. For these reasons, the results shown here are only suggestive and not conclusive.

We are convinced, however, that a repetition of our earlier study sponsored by CTR in 1960 to 61 would yield similar and conclusive evidence if indeed the composition of present day cigarettes has changed. We are planning the experiment summarized in Table II.

1003546828

Condensates could be prepared by Bio-Research Consultants in an identical manner for all cigarettes smoked. Condensates would be diluted with equal parts of acetone as in our previous studies.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Bioassay of Tobacco Smoke Condensates and Related Problems

By use of the ATC cigarettes (which we obtained from Dr. Hockett and understand to be made of the cigar tobacco used in our original 1960 experiment), we could obtain a check on the reproducibility of the first (1960) experiment and thereby determine that CAF_1 mice respond today in a manner similar to 1960. We could by this device obtain a comparison with the Millerton mice used by Wynder in most of his published studies. Inclusion of the two most widely smoked non-filter cigarettes of today is logical.

The use of primed animals having received 400γ of benzpyrene as initiator measures the co-carcinogenicity of the condensates, and the use of croton oil as a promotor yields early information on the carcinogenicity of the condensates.

This is an elaborate, complex and protracted (two years) experiment. It will provide conclusive evidence not obtainable by any other means and might well demonstrate the absence of co-carcinogenicity and greatly attenuated carcinogenicity of present day cigarettes.

In view of our latest skin-painting studies (Table I) such an outcome may be predicted as likely.

100354829

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Bio-Research Institute, Inc.

Table I

SUMMARY OF SOME RECENT MOUSE SKIN PAINTING EXPERIMENTS

Date of beginning of project	1961* -62	Jan. 1963	May 1964	July 1964	April 1965	Dec. 1965		Feb. 1966		August 1966	
Mice	CAF-1/Jax ♀♀	Millerton Swiss ♀♀	Charles River Swiss ♀♀	CAF-1/Jax ♀♀	Millerton Swiss ♀♀	CAF-1/Jax ♀♀				Millerton Swiss ♀♀	
Primed with benzo[a]pyrene†	-	-	+	-	+	+	-	-	-	-	+
Cigarette condensate applied:											
Per cent tar	50%	50%	50%	18.6%	50%	50%		18.6%	50%	18.6%	50%
Per cent water	-	-	-	15.4%	-	-		15.4%	-	-	
No. of mice per group at start	100	200	45	200	150	100	50	50	50	50	100 60
Papillomas after:											
30 weeks				0.5%							
40 "			56%		14%						
42 "	1%	10%	58%			8%	0%	0	2%	0	
57 "	9%			1%							
66 "	20%	39%		6%				0	7%	2%	
76 "	32%	60%		12%		21%	7%				

* Published J. Nat. Cancer Inst. 31:1445, 1963.

† 400μg per mouse.

1003546830

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Table II

PROPOSED EXPERIMENT FOR CTR MOUSE SKIN PAINTING

Group No.	Strain of Mice	No. Mice per Group	Treatment of Mice		
			Primer* (mg per mouse)	Cigarette Smoke Condensate Derived from**	Croton Oil
1	CAF/1	100	-	ATC†	-
2	"	100	-	Brand A	-
3	"	100	-	Brand B	-
4	"	100	-	none#	-
5	Millerton Swiss	100	-	ATC†	-
6	"	100	-	Brand B	-
7	"	100	-	none#	-
8	CAF/1	50	0.4	ATC†	-
9	"	50	0.4	Brand B	-
10	"	50	0.4	-	0.75
11	"	50	0.4	none#	-
12	"	50	-	-	0.75
Total		950			

* Benzo[a]pyrene
** All cigarettes of regular length (70 mm)
† All-tobacco cigarettes
# Acetone control

100354683

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

6. Budget Plan:*

Based on current level of endeavor.

a.	Salaries	34,760.
b.	Expendable Supplies	5,300.
c.	Other Expenses	3,085.
d.	Permanent Equipment	6,000.
e.	Overhead (15% of a, b, c)	6,472.
	Total	55,617.

* 2/1/68-1/31/69

7. Anticipated Duration of Work: Five years

8. Facilities and Staff Available: The same personnel as in the past will remain active in this work. In addition, a new group is being developed for studies on tissue cultures and hamster oncology in our new Cummington Street annex. It is anticipated that with addition of increased staff in the new division for work with hamsters, some of the mouse work will be reduced so that during each of the next five years, the budget for this project will remain approximately the same.

9. Additional Requirements: Condensates for the carcinogen inhibition and carcinogen acceleration studies will be provided by Bio-Research Consultants free of charge for labor costs only because their smoking machine was developed under CTR contract. In the case of skin-painting studies where large amounts of condensate are required, this will have to be purchased by CTR at the cost incurred by necessary addition of technical personnel for the production of these large amounts of condensate. However, it is anticipated that the new condensate machine will be so much more efficient than existing smoking machines that this cost will be small.

10. Additional Information (Including relation of work to other projects and other sources of support): In the past, it has been possible to share some personnel with our carcinogenesis studies carried out under National Institutes of Health, National Cancer Institute Research Grant No. CA-04869. Since this grant has been discontinued, this is no longer possible. Thus, support for these studies by CTR is assuming added importance for us. Even with it (at the requested rate), we must reduce our total effort in the carcinogenesis field. Without CTR support, we should have drastically to reduce our efforts in this field in which we have worked since 1948 and for which we have developed a uniquely competent team.

1003546832

Signature [signature]
Director of Project

[signature]
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/pfkh0000

#626 · REDISCH

1003546833

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 3, 1967

MEMORANDUM

Denied

TO: The committee comprising Dr. Bing, Chm., Dr. Cattell and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New application from Walter Redisch, M.D. - No. 626.

We enclose herewith an application for a research grant from Dr. Walter Redisch of New York University Medical Center, New York, N.Y.

We have supported Dr. Redisch's program for a number of years, as shown on the first page, but since the latest was designated a terminal grant, a new number has been assigned to the present application. It includes a comprehensive summary of work done under previous grants, which we requested.

Robert C. Hockett

1003546834

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

NO. 626

CF. #495
Activated: 7/1/66
CF. #344 - 1962-'64
COMPARE:
#33 - 1955 - 1957
#160 - 1957
#213 -1959 - 1961
#301 -1961 - 1962

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

COMMITTEE:
Dr. Bing, Chm.
Dr. Cattell
Dr. Jacobson

633 THIRD AVENUE
NEW YORK, N. Y. 10017

Application For Research Grant

Date: June 23, 1967

1. Name of Investigator(s): (include Title and Degrees) Walter Redisch, M.D., Assoc. Prof. Clin. Med., NYU School of Med.; Res. Assoc. NYU Res. Service, Goldwater Mem. Hosp., Welfare Island, N.Y.
E.N. Terry, M.D., Res. Fellow, NYU Res. Service, Goldwater Mem Hosp., Welfare Island, N.Y.

2. Institution & Address:
New York University Research Service, Goldwater Memorial Hospital, Welfare Island, N.Y.
New York University Medical Center, 550 First Avenue, New York, N.Y.

3. Short Title of Project:
~~Blood flow in patients with essential hypertension~~ (The effect of cigarette smoking on peripheral blood flow in patients with essential hypertension, untreated and treated)

4. Proposed Starting Date: October 1, 1967

5. Anticipated Duration of this Specific Study: One year

6. Brief Description of Objectives or Specific Aims:
It is suggested that the effect of cigarette smoking be studied and compared in the following groups:

1) patients with primary hypertension who have not been treated before and are not being treated while the study is in progress
2) patients with primary hypertension treated with reserpine
3) patients with primary hypertension treated with thiazide
4) patients with a primary hypertension treated with reserpine thiazide combination
5) patients with primary hypertension treated with guanethedin
6) normotensive subjects

Each group to consist of 5 subjects.

Essential hypertension for the purposes of this study is defined as a sustained diastolic pressure of 100 mm Hg or higher without detectable underlying cause.

7. Give a Brief Statement of your Working Hypothesis:

Appended A.

100354835

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

The method to be used in this study is differential venous occlusion plethysmography.

Plethysmographic measurements of the foot and calf are taken, those of the foot being representative of skin blood flow while measurements of the calf are representative of muscle blood flow. The calf and foot are placed in the appropriate plethysmographic lucite chambers. The chambers are then made airtight with vaseline and foam rubber. Volume changes of the limb are calculated from the effect of a known volume, namely 5 ml air injected into the chamber, has on the deflection of the recorder. On the recording a time-scale permits measurement of the distance equivalent to 10 seconds on the base line. At this point a perpendicular is erected. Blood flow in ml/minute in 100 cc of tissue is calculated from the formula:

$$\frac{600 \times L}{K \times V} = \text{Blood Flow (ml per minute per 100 ml of tissue)}$$

L is the perpendicular rise of the slope in mm, during 10 seconds. K is the calibration constant of the recording apparatus in mm deflection per ml of change in volume. V is the volume of the enclosed calf or foot in ml which is determined by the water displacement overflow method. (cont'd) Appended B.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

New York University Research Laboratories, Goldwater Memorial Hospital, Welfare Island, N.Y. Vascular Research Laboratory, New York University Medical Center, 550 First Ave., N.Y.

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

Appended D

12. List of publications: (Five most recent as pertinent) (append)

Appended C

10035468836

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Larry Rouen Research Technician	80%	$ R
Guillermo Cortes Research Technician	80%	
Employees Fringe Benefits		
Technical		
	Sub-Total	R
B. Consumable Supplies (list by categories)		
Mercury strain gauges ..... $325.		
Filters, gauge elements and other parts for Constant Temperature Room equipment which needs periodic replacements ..... 175.		
Batteries, elements, paper and ink for Speedomax and Plethysmograph recording 100	Sub-Total	1,000.
Photographic equipment for follow of microvasculature ..... 400.		
C. Other Expenses (itemize)		
Travel (domestic)		
To attend Scientific Meetings	Sub-Total	500.
D. Permanent Equipment (itemize)		
None		None
E. Overhead (15% of A+B+C)		2.191.
	Total	$16,795.

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature E. Walker Keeling
Director of Project
688-3500 Ext 507
Telephone

Signature
Business Officer of the Institution
Telephone

10035468837

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Vascular Research Fund	SKF and Arlidin	4,000.	Indef.
	Role of Catecholamines in Vasoconstriction	USPHS	30,358.	6/1/67-5/31/68
	Microcirculation in Systemic Rheumatic Disease	USPHS	10,421. to ---	9/1/67
Pending				

100354838

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

A

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

Application For Research Grant

Dr. Walter Redisch
New York University School of Medicine
New York University Research Service

7. Give a Brief Statement of your Working Hypothesis:

There is no proof that cigarette smoking and tobacco smoking in general have the same effects on various physiologic parameters. There is likewise no definite proof that these effects are due to nicotine. However, the effects of cigarette smoking on the cardio-vascular system show some essential similarities to the pharmacologic effects of nicotine.

The pharmacologic action of nicotine consists of a primary transient stimulation and secondary more persistent depression of all sympathetic and parasympathetic ganglia. This includes the adrenal medulla and epinephrine is thereby discharged. Nicotine and tobacco smoking have also been shown to exert an antidiuretic action. This effect is thought to be the result of stimulation and of the supraoptical-hypophyseal system with the subsequent release of posterior pituitary anti-diuretic hormones. Nicotine also markedly stimulates the central nervous system which is particularly evident for the respiratory and vasomotor centres of the medulla. Due to the multiple sites of action one may encounter initially a slowing of the heart rate due to stimulation of the central vagal nuclei and cardiac vagal ganglia. Later owing to the stimulation of sympathetic ganglia, and the central vasomotor centre, tachycardia and a peripheral vasoconstriction may become prominent.

After the stage of sympathetic ganglionic stimulation is succeeded by paralysis, smaller vessels of the periphery, especially those of the skin may dilate.

These responses should be studied to ascertain the presence or absence of an increased catecholamine activity in the systemic blood vessels of patients with hypertension and the presence or absence of a basic abnormality in catecholamine metabolism in primary hypertension. Such findings in untreated patients with primary hypertension should be compared to findings in patients treated with various drugs to lower blood pressure. Also the effect of cigarette smoking on the treatment of hypertension with diuretics should be studied.

1003546839

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

B

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

Application For Research Grant

Dr. Walter Redisch
New York University School of Medicine
New York University Research Service

8. Details of Experimental Design and Procedures: (cont'd)

When measuring blood flow through the calf (i.e. muscle vascular bed) two pressure cuffs are applied to the extremity to be measured. The distal (occlusion) cuff is inflated to 270 mm Hg, the one proximal to the plastic case (collecting cuff) to the individual's diastolic blood pressure minus 10 mm Hg. When measuring blood flow to the foot (i.e skin vascular bed) only the collecting cuff is used which is inflated to the individual's diastolic blood pressure minus 10 mm Hg. Following sudden occlusion, the linear portion of the rising slope of the recorded curve is used to calculate the rate of flow according to the formula mentioned above.

All experiments of course will be done in a constant temperature laboratory, at temperatures of 20 and 28°C. Humidity will be held constant at 55%.

In order to avoid environmental influences, patients in basal state will be brought to the constant temperature laboratory where they will rest on a bed while quasi-continuously the skin temperature on both big toes and the right middle finger are recorded using a six channel Speedomax. A patient is considered to be adjusted to the environment of the room when the skin temperature of his toes and fingers has come down to constant room temperature and stayed there for thirty minutes. In patients whose skin temperature does not come down to room temperature despite prolonged exposure, maintenance of a constant skin temperature of toes and fingers for 30 minutes is considered to represent adaptation.

For smoking, our standard procedure will be used, subjects will be instructed and supervised to smoke at the rate of one inhalation every thirty seconds for six minutes (total of twelve inhalations).

1003546840

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

C

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

Application For Research Grant

Dr. Walter Redisch
New York University School of Medicine
New York University Research Service

12. Publications

Studies in Tobacco Hypersensitivity.
Fontana, V.J., Redisch, W , Nemir, R., Smith, M.K., DeCrinis, K. and Sulzberger, M.B.
J. of Allergy, 30: 241, 1959.

Tobacco Hypersensitivity: Peripheral Circulatory Implications.
Walter Redisch, M.D.
Presented at the New York Academy of Sciences, March 25, 1960.
Annals, N.Y. Acad. of Sciences 90: Art. 1, 142-144, Sept. 27, 1960.

Vascular Responses to Smoking Tobacco Compared with Responses to Skin Testing of Tobacco Extracts.
DeCrinis, K., Redisch, W., Fontana, V., Lewis, A., Sulzberger, M.B. and Steele, J.M.
Annals Int. Med. 52: No. 5, 1960.

Tobacco Allergy and Vascular Responses.
Redisch, W.
Reprinted from BOOK - TOBACCO AND HEALTH, James and Rosenthal, et al..
Charles C. Thomas, Publisher, Springfield, Illinois, pp. 352, 1962.

Studies on Effects of Catecholamines Upon Extremity Blood Flow in Man.
Redisch, W.
Metabolismus Parietis Vasorum - Praha Diebus - 4 - 9 September 1961.

Evaluation of Vascular Responses to Cigarette Smoking.
Redisch, W., Messina, E.J., Terry, E.N., Rouen, L.R. and Steele, J.M.
Submitted for publication, 1967, Angiology. (Accepted 7/27/67)

A Manifestation of Diabetic Microangiopathy in Nailfold Capillaries.
Terry, E.N., Messina, E.J., Schwartz, M.S., Redisch, W. and Steele, J.M.
Diabetes, in press.

Blood Flow Measurements in Response to Bamethane Sulfate in Man.
Terry, E.N., Messina, E.J., Redisch, W. and Steele, J.M.
Angiology 18: 161-173, March 1967.

100354841

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

D

CURRICULUM VITAE

Name: Walter Redisch, M.D., F.A.C.P., F.R.S.A.

Address: R

Date and Place of Birth: R Prague, Bohemia

Marital Status: R

Former Appointments:

Demonstrator, Dept. of Physiol., German University of Prague	1921-38
Fellowship, Heart Station, Vienna University	1927
Fellowship, Dept. of Internal Medicine, Franz Joseph's Hospital, Vienna	1929
Fellowship, Dept. of Internal Medicine, University of Tuebingen, Tubingen, Germany	1924
University Ass't., Dept. of Gen. and Exp. Path. and Clin. Propedeutics, German University Med. School of Prague and Univ. Hospital	1923-32

Present Appointments:

Associate Professor of Clinical Medicine, New York University School of Medicine, New York, NY

Research Associate, New York University Research Service, Goldwater Memorial Hospital, New York, NY (Director, Dr. J. Murray Steele)

Visiting Physician, New York University Medical Research Division, Goldwater Memorial Hospital, New York, NY

Associate Visiting Physician, Bellevue Hospital, New York, NY

Associate Attending Physician, University Hospital, New York, NY

Physician-in-charge, Vascular Section, New York University Cardiovascular Clinic, Bellevue Hospital.

Physician-in-charge, Medical-Surgical Vascular Group, New York University Medical Center (Surgeon-in-charge, Dr. Roy Clauss)

Member, Advisory Board, Council on Circulation, A.H.A.

Consultant, Vascular Disease, St. Michael's Hospital, Newark, New Jersey.

10035468 42

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Curriculum Vitae
Walter Redisch, M.D.

War Activities: Participated in a project for the United States Navy of the Office of Scientific Research and Development under the direction of Dr. Steele . . . 1941-42

Military Service: Major, USA Medical Corps first on New Guinea and then Chief of Medical Service, Regional Hospital, Camp Shelby, Mississippi 1943-46

During 37 months of war service on official leave of absence from the University.

Member of:

REDACTED REDACTED

REDACTED REDACTED

10035468843

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

D

CURRICULUM VITAE

NAME:	Erwin N. Terry, M.D.	
ADDRESS:	R	
DATE AND PLACE OF BIRTH:	R Vienna, Austria	
MARITAL STATUS:	R	
EDUCATION:	Tel-Aviv High School of Commerce, Palestine	1940
	No. 7 Formation College	1945-46
	Medical Faculty of the University of the Saar	1951-56
MILITARY SERVICE:	Major, British Army and Israel Defense Forces	1941-46
FORMER APPOINTMENTS:	Moniteur, University of Saar, Department of Psychiatry	1954-56
	Civilian Contract Physician, P.A.C. Hospital, U.S. Air Force in Germany	1956-61
	Associate Medical Director, C.H. Boehringer Sohn, Ingelheim/Rhein, Germany	1961-63
PRESENT APPOINTMENTS:	Medical Director, Pharma Research Canada, Ltd., Pointe Clair, Quebec	1963-
	Research Fellow, New York University Research Service, Goldwater Memorial Hospital, Welfare Island, NY, NY	1964-
MEMBER OF:		

REDACTED REDACTED REDACTED

10035468844

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Comprehensive Report

The Council for Tobacco Research

Walter Redisch, M.D.
Associate Professor of Clinical Medicine, New York University School of Medicine.
Research Associate, New York University Research Service, Goldwater Memorial Hospital

BLOOD FLOW STUDIES IN HYPERTENSIVE SMOKERS

This laboratory has studied vascular responses to cigarette smoking in man since 1955. There have been 4 phases of the investigation so far.

1) 1955 - 1959

In cooperation with Drs. Marion Sulzberger and Vincent Fontana it was first ascertained that in healthy volunteers the percentage of subjects with strongly positive reactions to skin testing with tobacco extract was about the same as the percentage of subjects who showed a significant decrease in blood flow response to cigarette smoking. The effects of cigarette smoking on blood flow in the lower extremity measured by plethysmography were then ascertained in 80 healthy subjects and correlated to other hemodynamic parameters as well as to the results of testing for skin sensitivity to tobacco extracts. Comparison with skin testing revealed on significant correlation: 90% of those who did not react to skin testing with tobacco extract had no decrease in peripheral blood flow in response to smoking.

2) 1959 - 1962

Since blood flow responses to smoking in the lower extremity seemed erratic, in that some test subjects showed unequivocal increase in total flow, it soon became evident that it was necessary to employ some acceptable means of separately estimating skin flow and muscle flow. A method of differential plethysmography has been developed in our laboratory on the basis of the rather constant difference between skin-mass/muscle mass ratio in the foot and in the leg. The formula following permits a rough estimation of skin and muscle flow by correcting for the most obvious errors.

$$Fl + Ff = F \text{ (c.c./min.)}$$
$$Vl + Vf = V \text{ (c.c.)}$$
$$P = \frac{F}{V} \text{ (c.c./100 c.c./min.)}$$

$$Ps = Pf + Cf\,\Delta \qquad Cf = 0.20$$
$$Pm = Pl - Cl\,\Delta \qquad Cl = 0.26$$
$$\Delta = \frac{Pf - Pl}{1 - Cf - Cl}$$

F = Flow Rate V = Volume
P = Perfusion Rate
L = Leg F = Foot S = Skin
M = Muscle C = Correction Factor

Of course, all experiments are done in the Constant Temperature Laboratory; the ones reported here were done at 25°C and 55% humidity.

1003546845

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

The Council for Tobacco Research

In order to be reasonably sure that the differences be meaningful, we considered as significant only changes of more than 1 1/2 ml. per 100 ml. of tissue/min.

At the present time 34 subjects have been tested with this method. There were 25 males and nine females. Their ages ranged from 24 to 87 years with an average of 56.3. Seven of the 34 subjects exhibited evidence of obliterative arteriosclerosis, while the rest had no demonstrable vascular disease. Nine of the group were significantly sensitive to one or more specific tobacco extracts. Skin and muscle flow responses of these 9 subjects were tested for the smoking of tobacco types to which their skin was sensitive.

		Skin Flow			Muscle Flow		
		Decrease	No Change	Increase	Decrease	No Change	Increase
Total Allergic	9	8	1	0	2	3	4
No OAS	8	7	1	0	2	2	4
OAS	1	1	0	0	0	1	0

In response to smoking, in eight of these nine tobacco-sensitive persons a significant decrease in skin blood flow was observed. One had a decrease not significant according to our criteria and was therefore listed as showing "no change"; none had an increase in skin flow.

		Skin Flow			Muscle Flow		
		Decrease	No Change	Increase	Decrease	No Change	Increase
Total Non-Allergic	25	2	15	8	2	12	11
No OAS	19	1	10	8	2	9	8
OAS	6	1	5	0	0	3	3

In contrast, of the 25 subjects who were skin negative, eight showed an increase in skin flow, 15 had no change and in only two a significant decrease in skin flow was observed.

There was no conclusive trend in muscle flow changes in either group. The differences in skin flow responses are highly significant.

3) 1962 - 1964

The study now turned to patients with non-gangerous occlusive atherosclerosis. 22 patients were studied in more than 100 experiments. The results

1003546846

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

were by and large identical with those in healthy subjects.

4) 1964 - 1967

The last phase of the project was devoted to a critical re-evaluation. The following conclusions were reached:

On the basis of studies carried out using segmental venous occlusion plethysmography "opthalmic artery pulsensor", capillaroscopy and photography and skin sensitivity testing for tobacco extracts, the following conclusions seem justified:

1) Vascular response to cigarette smoking in the lower extremity differs in skin and muscle. A decrease in skin perfusion is generally accompanied by an increase in muscle perfusion and vice versa.

2) Changes in relationship between skin and muscle perfusion depend quantitatively and qualitatively on environmental temperature.

3) At "neutral" temperatures between 22.5°C to 23.5°C., vascular responses to cigarette smoking are quantitatively at their lowest.

4) Whenever skin perfusion is decreased at 20°C following cigarette smoking, it will show an increase in response to cigarette smoking 25°C. Whenever muscle perfusion is increased in response to cigarette smoking at 20°C, it will decrease at 25°C in response to the same stimulus.

5) There is almost constant relationship between skin and muscle perfusion in vascular responses to smoking in the lower extremity. As a rule, increase in one is accompanied by a decrease in the other and vice versa.

6) Microcirculatory changes in response to tobacco smoking are dynamic in nature. The analysis of these changes require microcineamatography because of the rapid rate at which they occur.

7) "Opthalmic artery pulsensor" studies do not seem to be of value in assessing the response to smoking.

8) Skin sensitivity reaction to tobacco extracts may be dependent to a very large extent on preparation and composition of the extract.

1003546847

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Total Essay of Proposal

BLOOD FLOW STUDIES IN HYPERTENSIVE SMOKERS

The effects of cigarette smoking on the peripheral circulation have been studied extensively in healthy subjects and in patients with arteriosclerosis. No reference could be found to a study of effects of cigarette smoking on blood-flow in patients with hypertension, untreated or under treatment with various substances.

In primary hypertension, the elevation of blood pressure is considered to be due to an increased peripheral resistance in the circulatory system. Until recently it was thought that cardiac output and stroke volume in primary hypertension are within normal limits. There was little or no evidence for over-activity of the sympathetic nervous system, vasoconstrictive reflexes remaining at normal levels. In the majority of cases catecholamine excretion, as a measure of sympathetic activity, was found to be within normal limits.

In the early labile stage of primary hypertension, some moderate elevation of catecholamine excretion has been shown in some cases. This elevation was particularly evident during peak elevations of blood pressure.

The whole concept of peripheral vascular resistance is ill defined. It has been shown that circulation in various organs or vascular beds may respond differently to a different stimulus. It has also been demonstrated that the ststus of a particular vascular bed, prior to stimulation, will influence the subsequent response. Observing vascular responses in one vascular bed does not necessarily permit the prediction of similar responses in other areas This concept of variability of vascular response in differing vascular beds would appear to contradict the concept of increase in total peripheral vascular resistance in essential hypertension.

A number of drugs currently in use to lower blood pressure affect catecholamine function in one way or another. Thus the question has been raised whether in primary hypertension one is basically dealing with an abnormality in catecholamine metabolism. Though it would appear that overall synthesis of catecholamines and their release from storage by neurone discharge as judged by urinary catecholamine excretions is normal, it has been shown that systemic blood vessels of subjects with hypertension are more reactive to catecholamines such as Norepinephrine than those of normals. This then, would suggest that the Norepinephrine released in response to nerve discharge is modulated differently. It has also been suggested that a defect of the storage mechanism of Norepinephrine, being smaller in hypertension compared to the normal, would lead to a greater response to infused or endogenous Norepinephrine as well will be absorbed, leaving more available for vasoconstriction. A third possibility to explain the increased vascular reactivity in primary hypertension has been suggested, namely insufficient tissue binding of exogenous or endogenous norepinephrine thus leading to an exaggerated vasoconstrictor response.

The pharmacologic action of nicotine consists of a primary transient stimulation and secondary more persistent depression of all sympathetic and parasympathetic ganglia. This includes the adrenal medulla and epinephrine

1003546848

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

is thereby discharged Nicotine and tobacco smoking have also been shown to exert an antidiuretic action. This effect is thought to be the result of stimulation of the supraoptical-hypophyseal system with the subsequent release of posterior pituitary anti-diuretic hormones.

Nicotine also markedly stimulates the central nervous system which is particularly evident for the respiratory and vasomotor centres of the medulla. Due to the multiple sites of action one may encounter initially a slowing of the heart rate due to stimulation of the central vagal nuclei and cardiac vagal ganglia. Later owing to stimulation of sympathetic ganglia, and the central vasomotor centre, tachycardia and a peripheral vasoconstriction may become prominent.

After the stage of sympathetic ganglionic stimulation is succeeded by paralysis, smaller vessels of the periphery, especially those of the skin, may dilate.

These combined effects of nicotine should be studied to ascertain the presence or absence of an increased catecholamine activity in the systemic blood vessels of patients with hypertension and the presence or absence of a basic abnormality in catecholamine metabolism in primary hypertension. Such findings in untreated patients with primary hypertension should be compared to findings in patients treated with various drugs to lower blood pressure. Also the effect of smoking on the treatment of hypertension with diuretics should be studied.

It is suggested that the effect of cigarette smoking be studied and compared in the following groups:

1) patients with primary hypertension who have not been treated before and are not being treated while the study is in progress

2) patients with primary hypertension treated with reserpine

3) patients with primary hypertension treated with thiazide

4) patients with a primary hypertension treated with a reserpine thiazide combination

5) patients with primary hypertension treated with guanethedin

6) normotensive subjects

Each group to consist of 5 subjects
Essential hypertension for the purposes of this study is defined as a sustained diastolic pressure of 100 mm Hg or higher without detectable underlying cause.

1003546849

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Blood Flow Studies in Hypertensive Smokers

The method used in this study is differential venous occlusion plethysmography.

Plethysmographic measurements of the fot ad calf are taken, those of the foot being representative of skin blood flow while measurements of the calf are representative of muscle blood flow. The calf and foot are placed in the appropriate plethysmographic lucite chambers. The chambers are then made airtight with vaseline and foam rubber. Volume changes of the limb are calculated from the effect of a known volume, namely 5 ml air injected into the chamber, has on the deflection of the recorder. On the recording a time-scale permits measurement of the distance equivalent to 10 seconds on the base line. At this point a perpendicular is erected. Blood flow in ml/minute in 100 cc of tissue is calculated from the formula:

$$\frac{600 \times L}{K \times V} = \text{Blood Flow (ml per minute per 100 ml of tissue)}$$

L is the perpendicular rise of the slope in mm, during 10 seconds. K is the calibration constant of the recording apparatus in mm deflection per ml of change in volume. V is the volume of the enclosed calf or foot in ml which is determined by the water displacement overflow method. When measuring blood flow through the calf (i e. muscle vascular bed) two pressure cuffs are applied to the extremity to be measured. The distal (occlusion) cuff is inflated to 270 mm Hg, the one proximal to the plastic case (collecting cuff) to the individual's diastolic blood pressure minus 10 mm Hg. When measuring blood flow to the foot (i e. skin vascular bed) only the collecting cuff is used which is inflated to the individual's diastolic blood pressure minus 10 mm Hg. Following sudden occlusion, the linear portion of the rising slope of the recorded curve is used to calculate the rate of flow according to the formula mentioned above.

All experiments will of course be done in a constant temperature room, at temperatures of 20 and 28°C. Humidity to be held constant at 55%.

In order to avoid environmental influences, patients will be brought to the constant temperature laboratory where they will rest on a bed while quasi-continuously the skin temperature on both big toes and the right middle finger are recorded using a six channel Speedomax. A patient is considered to be adjusted to the environment of the room when the skin temperature of his toes and fingers has come down to constant room temperature and stayed there for thirty minutes. In patients whose skin temperature does not come down to room temperature despite prolonged exposure, maintenance of a constant skin temperature of toes and fingers for 30 minutes is considered to represent adaptation.

Walter Redisch, M.D.
New York University School of Medicine.

1003546850

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#631--DOMINO

1003546851

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

NO. 631

Cf. #206

Activated: 1/1/59

Renewed annually on the anniversary date through 1/1/67.

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Jacobson
Dr. Little

Application For Research Grant

Date: July 21, 1967

Approved.

1. Name of Investigator(s): (include Title and Degrees)

Edward F. Domino, M.S., M.D., Professor of Pharmacology

2. Institution & Address: University of Michigan
Department of Pharmacology
6440 Medical Science Bldg.
Ann Arbor, Michigan 48104

3. Short Title of Project:

Effects of Tobacco Smoking and Nicotine on the Central Nervous System

4. Proposed Starting Date: January 1, 1968

5. Anticipated Duration of this Specific Study: Three years. Phase "a" should be completed within 1 year.

6. Brief Descripton of Objectives or Specific Aims:

a. To determine effects of inhaling tobacco smoke of high and low nicotine containing cigarettes on skeletal muscle tone in animals and man.

b. To compare these effects with i.v. nicotine injections.

c. To further delineate the CNS actions of nicotine.

7. Give a Brief Statement of your Working Hypothesis: People smoke tobacco for the psychopharmacological effects of nicotine. These effects are identifiable and measurable using objective endpoints of central nervous system action.

1003546852

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

Our research approach has been oriented to the question of why people smoke tobacco. To date this research has dealt with the behavioral arousal effects of nicotine and tobacco smoking. These effects are very mild and are accompanied by a unique relaxing effect. This unique muscle relaxant effect has been observed previously by others as described in "Tobacco: Experimental and Clinical Studies" by Larson, Haag and Silvette. Many of the previous studies have used large toxic doses of nicotine so that the relationship of data so obtained to tobacco smoking is unclear. Furthermore, it is our hypothesis that the pharmacologically active substance in tobacco, i.e., nicotine, acts mostly on the central nervous system in causing a reduction in skeletal muscle tone. Most of the known peripheral effects of small doses of nicotine are to increase skeletal muscle tone. The proposed research will be conducted in two phases.

1. Effects of tobacco smoking of high and low nicotine content on skeletal muscle tone.

2. Effects of nicotine given intravenously on skeletal muscle tone.

Standard techniques will be used to evaluate the skeletal muscle effects of nicotine and tobacco smoking. These have been published in detail by the principal

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location) (Cont'd. p. 2a).

Department of Pharmacology
6440 Medical Science Building

10. Additional Requirements:

Cigarettes of known tar and nicotine content are desired. These must look identical and hopefully taste somewhat similar. It is estimated about two cartons of each type would be required.

11. Biographical sketches of all principal and professional personnel (append).

See appended vita of Drs. Domino, Miyasaka and Nakai

12. List of publications: (Five most recent as pertinent) (append)

10035468S3

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

invest.gator (see N. Y. Acad. Sci. 64: 705-729, 1956 and more recently, Centrally Acting Skeletal Muscle Relaxants, Chapt. 15, 313-324 in Evaluation of Drug Activities: Pharmacometrics, Vol. 1. D.R. Lawrence and A. L. Bacharach, Eds. Academic Press, Lond, 1964). In man the EMG and patellar reflex will be measured in response to fixed muscle tensions. This will be done before, during, and after tobacco smoking of cigarettes of known nicotine content as phase I of this project. A total of 10 smokers and 10 former or occasional smokers will be studied. Each will be given 2 cigarettes to smoke at each recording session. Each cigarette will be smoked over a fixed period of 3 minutes during which muscle activity will be recorded. At weekly intervals the subjects will be given a cigarette of high, low, zero nicotine content and a sham smoke in a Latin square design.

During phase II the study will switch to the use of intravenous nicotine over a 5 minute period to simulate the amount inhaled from the high nicotine content cigarette. A maximal dose of 10-20 microgm/kg will be given depending upon tolerance, etc.

Similar studies will be conducted in the chloralose anesthetized cat using this as a model system to determine more precisely the site of action of nicotine.

In addition our previous studies (see Progress Report #9) on the effects of nicotine on the visual sensory input and its modulation will be continued as described previously. The correlation of nicotine content in the brain with its neurological and behavioral effects will be studied as soon as a supply of ^{14}C-labelled nicotine becomes available to us through Dr. Armitage of England.

1003546854

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
M. Miyasaka, M.D. (Postdoctoral Fellow)	100	R
Y. Nakai, M.D. (Postdoctoral Fellow)	100	
Technical		
Sub-Total		R
B. Consumable Supplies (list by categories)		
Animal		2000
Surgical		1000
Chemical		250
Glassware		500
Photographic		500
Polygraph recording paper		750
Sub-Total		5000
C. Other Expenses (itemize)		
12% Staff benefits for personnel		1800
Student Volunteers		1000
Travel to yearly Pharmacology Meetings		300
Sub-Total		3100
D. Permanent Equipment (itemize)		
Infusion pump		400
Strain gauge, amplifier and coupler		400
Respirator		500
EMG amplifier and integrator		700
		2000
E. Overhead (15% of A+B+C)		3465
Total		28,565

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	5250	3200	2000	3307	29,507
Year 3		5512	3300	2000	3472	30,821

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Edward A. Dennis
Director of Project

Telephone

Signature
Business Officer of the Institution

Telephone

10035468S5

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
CNS Actions of Substances Implicated as Neurohumors	NINDB	$19,680	9-1-66 to 8-31-67

Pending

The above grant will terminate 8-31-67. A competing renewal grant is being submitted with a May 1, 1968 starting date. However, even if approved this grant only is indirectly related to the tobacco and nicotine project as it involves central cholinergic mechanisms since nicotine is not a neurotransmitter substance.

100354856

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

BIOGRAPHICAL SKETCHES

Name:	Title:	Birthdate:
Matué Miyasaka	Postdoctoral Fellow	R

Place of Birth:	Present Nationality:	Sex:
Suwa City, Nagano Prefecture Japan	Japanese	Male

EDUCATION

Institution:	Degree:	Year:
Tokyo Medical and Dental University	M.D.	1955
Tokyo Medical and Dental University	D.Med.Sc.	1960

Major Research Interest:

Relationship to Project:

Postdoctoral Fellow

Research and Professional Experience:

Rotating internship, First National Hospital of Tokyo	1955-1956
Postgraduate Course, Department of Neuro-psychiatry	1956-1960
Official staff, Neuropsychiatric Department, Tokyo University	1960-1965
Postdoctoral Fellow, Department of Pharmacology, University of Michigan	1966-present

Publications:

Twenty-seven major publications have been published in various medical and scientific journals.

10035468572

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

BIOGRAPHICAL SKETCHES

Name:	Title:	Birthdate:
Edward F. Domino	Professor of Pharmacology	R

Place of Birth:	Present Nationality:	Sex:
Chicago, Illinois	U S Citizen	Male

EDUCATION

Institution:	Degree:	Year:
University of Illinois, Urbana, Illinois	B.S.	1948
University of Illinois, Chicago, Illinois	M.S. (Pharmacology)	1951
University of Illinois, Chicago, Illinois	M.D. (With honors)	1951

Major Research Interest:

Experimental and Clinical Neuropsychopharmacology

Relationship to Project:

Principal Investigator

Research and Professional Experience:

Professor of Pharmacology, The University of Michigan	1962 to present
Associate Professor of Pharmacology, The University of Mich.	1958-1962
Assistant Professor of Pharmacology, The University of Mich.	1954-1958
Instructor, Pharmacology, The University of Michigan	1953-1954
Instructor, Pharmacology, University of Illinois	1952-1953
Rotating Internship, Presbyterian Hospital, Chicago	1951-1952

Publications:

Over 160 full-length manuscripts and abstracts on neuro- and psychopharmacology have been published in various scientific journals.

10035468S8

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

[redacted]

BIOGRAPHICAL SKETCHES

Name:	Title:	Birthdate:
Yoshihisa Nakai	Postdoctoral Fellow	R
Place of Birth:	**Present Nationality:**	**Sex:**
	Japanese	Male

EDUCATION

Institution:	Degree:	Year:
Kyoto University, Kyoto, Japan	B.S.	1955
Kyoto University, Kyoto, Japan	M.D.	1959
Kyoto University, Kyoto, Japan	D.Med.Sc.	1965

Major Research Interest:

Sensory evoked responses

Relationship to Project:

Postdoctoral Fellow

Research and Professional Experience:

Internship at Kyoto University Hospital	1959-1960
Residency at Kyoto University Hospital	1960-1962
Postgraduate course at the Department of Pharmacology, Faculty of Medicine at Kyoto University	1961-1965
Postdoctoral Fellow, Department of Pharmacology, University of Michigan	1966-present

Publications:

Seven major publications have been published covering various aspects of the neuropharmacology of psychoactive drugs.

10035468859

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#632 STRONG

1003546860

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

August 28, 1967

Defer for Seattle's investigation

MEMORANDUM

TO: The committee comprising Dr. Reimann, Chm., Dr. Bing, Dr. Cattell and Dr. Sommers.

FROM: Robert C. Hockett

SUBJECT: New grant application from Jack P. Strong, M.D. - #632.

We enclose herewith a new grant application from Dr. Jack P. Strong of the Louisiana State University Medical Center in New Orleans, Louisiana. Veteran members of the Board will recognize this as a proposal to continue, without essential change, the autopsy study of the degree of sclerosis of certain arteries as it may be correlated with a few selected life-history factors on which information is collected retrospectively from "next-of-kin" after decease. These factors include smoking history.

The project, in an earlier and somewhat different phase, was started originally under Dr. R.L. Holman in 1958 and continued, first under Dr. H.C. McGill and then under Dr. J.P. Strong for a total of eight years. In line with our policy of trying to limit implied commitments to some realistic time basis, we asked Dr. Strong in 1963 to make an estimate as to how much longer the study would need to be continued to make possible statistically significant conclusions. His estimate was that two more years would be needed from February 1, 1964. Our budget projections were made on that basis, and a sixth renewal grant was approved to be effective on February 1, 1964 with one additional year of priority consideration promised. In accordance with this, a seventh renewal was approved, effective on February 1, 1965, with notification that this completed commitments of the Council and any further application would be considered "de novo" in the light of the current situation, without priorities.

In this last application Dr. Strong stated, "we wish to continue the study another year in accordance with the plan outlined in our last application. At the present rate of case completion, another year should be sufficient for the collection of the estimated minimum number [my underscoring] of cases needed in most of the major sub-classifications. Part of an additional year will be required for processing the interview data, completing the evaluation of the arterial specimens, and for conducting the definitive analysis of the data."

The SAB authorized an extension of the project, without additional funds, from February 1, 1966 to August 1, 1966 for some of this data processing. The project then terminated technically as of the latter date and a final comprehensive progress report and financial accounting are due. (The last financial accounting was received on April 9, 1964 covering the period from February 1, 1963 to January 31, 1964). A "final" progress report will no doubt depend on the completion of the interim data analysis mentioned in the application on page 2-a. Meanwhile addition of cases has continued.

1003546861

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

MEMORANDUM - CONT'D-

A first public report on the study was made at the October 19-20, 1966 Annual Meeting of the American Heart Association. An abstract of the paper presented at that time is attached. Also attached is a copy of the letter of comment I wrote to Dr. Strong on receipt of this abstract and a copy of the release by the American Heart Association on the day of presentation. Persons present at the meeting reported that he did state on the platform that the association he observed between smoking history and the degree of atherosclerosis could not be interpreted as showing that the smoking per se was responsibile for speeding the atherosclerotic process. (See attached memo of J.M.B. and my memorandum to the Board dated July 18, 1961).

Meanwhile there were newspaper reports of studies being conducted by Drs. McGill and Strong on the induction of atherosclerosis in primates. Following preliminary correspondence on this subject (copies enclosed) I made a visit to Dr. Strong on January 4, 1967 to review the project work and learn more about his animal studies. He told me about their comparative atherosclerosis studies in baboons, rhesus monkeys and squirrel monkeys both in the wild state and in captivity. They believe that the small squirrel monkeys may turn out to be a good animal model for atherosclerosis work. In the wild state they show no lesions, but in captivity with limited activity and on a diet of what they call their basic "monkey chow" with added butter, cholesterol and coconut oil, arterial lesions appear that are anatomically and pathologically very similar to human ones and can be scored for severity by methods very similar to those in use by Strong's group for scoring human autopsy material. The monkeys, unlike dogs, do not have to be made hypothyroid for induction of lesions.

My letter of May 9, 1967 comments further on some of the topics I discussed with Strong. I had rather expected that the next proposal we received would suggest controlled experiments with small primates to determine whether chronic nicotine absorption superimposed upon a tested atherogenic regimen would influence the degree of atherosclerosis resulting. Instead we have received the proposal for extension of the autopsy study.

(Mrs. Richards has been the interrogator in this project since the days of Dr. Holman. He and his successors all think that she is exceptionally good at this and Dr. Strong evidently wants to keep her on the job. The main item in our grants has been provision for her salary. They will argue that if further cases are to be added, this should be done now while the personnel and procedures are ready for the task. It does seem that if any job is worth doing at all, it is worth doing well enough to yield definite results and this study probably ought to be rounded out by somebody.

PERSONAL COMMENT:

I cannot judge from any data I have seen as to how significant Strong's reported association between smoking history and severity of atherosclerotic lesions really is. An extension of the study to more cases might confirm the association more definitely or conceivably diminish the strength of the correlation. If the association vanished, definite conclusions could be drawn. If it persisted, we would remain where we are -

1003546862

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

MEMORANDUM - CONT'D.

in a state of uncertainty as to whether nicotine and/or smoking actually do contribute causally to the progression of arteriosclerosis. Controlled animal experiments with chronic nicotine or smoking exposure and studies of how other characteristics and life practises cluster with smoking statistically, might provide better clues toward a solution of this key question and thus be a better investment.

R. C. H.

1003546863

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 28, 1967

JACK P. STRONG, M.D.

GRANTS TO PROJECT #174

#174 - February 1, 1958 - February 1, 1959	$7,705.00	
#174R1 - February 1, 1959 - February 1, 1960	7,705.00	
#174R2 - February 1, 1960 - February 1, 1961	7,705.00	(+ 3,500.00 SUP)
#174R3 - February 1, 1961 - February 1, 1962	14,292.00	
#174R4 - February 1, 1962 - February 1, 1963	14,989.00	
#174R5 - February 1, 1963 - February 1, 1964	15,822.00	
#174R6 - February 1, 1964 - February 1, 1965	15,775.00	
#174R7 - February 1, 1965 - February 1, 1966	17,531.00	
TOTAL	$105,024.00	

10035468864

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

McGill Copy.

cc: Drs. Little B
Wilson

July 23, 1951

MEMORANDUM

TO: The Subcommittee on Project #174 (Strong and McGill).
Drs. Cattell, Bing and Lynch.

FROM: Robert C. Hockett

An application for continued support has reached us from Dr. Henry C. McGill although his colleague, Dr. Strong, has now been named as Principal Investigator. This is the study of the degree of atherosclerotic disease as determined at autopsy, principally of accident victims, for correlation with habits of life, principally smoking. Although the anniversary date is not until February 1, 1952, this renewal application has been submitted early to ascertain the intentions of the Board well in advance.

In renewing the grant at the December 1950 meeting, the Board promised only one year of support, and agreed that a conference should be held in an effort to clarify a number of questions that might bear upon our future plans.

These questions included:

(1) The accuracy with which atherosclerotic lesions can be graded.
(2) The reliability of information that can be obtained on the life habits of deceased people.
(3) Whether the lesions in other parts of the vascular system than the coronary arteries should be included in the study.
(4) Whether other research groups are engaged in similar work so that larger bodies of comparable data could be obtained by cooperation, and
(5) What kinds of conclusions should be possible if the data could be collected and the correlations made.

There may have been others.

The conference on atherosclerosis studies was held on May 26, 1951 with representatives from eight or nine different research groups present. Most of the members of the subcommittee attended.

I believe that Dr. McGill's feeling is that his methods for rating atherosclerotic lesions are satisfactory for his purposes and I think he feels that this also was the consensus of the group. He feels also, I think, that his pilot studies on determining smoking habits from close associates of living subjects, as verified by interrogating the subjects themselves, has justified his confidence in the data he can get on this subject from close associates of persons now deceased. He believes that he can do equally well in getting data on alcohol consumption and

1003546865

Source: https://www.industrydocuments.ucsf.edu/docs/ptdn0000

he recognizes dietary information as equally or more important, he sees this as much more difficult and does not include it in his present plan, though the matter is under continuing study by his colleagues at the University of Louisiana.

With respect to other parts of the vascular system, he has a colleague who is interested particularly in the cerebral circulation and hopes to develop a collateral study of this system. This, however, is not included in his proposal to us, which is confined to study of the coronary arteries.

The conference did not reveal any other studies now underway with use of techniques sufficiently similar to suggest that any pooling of data would be possible.

The subcommittee must, therefore, reach a judgment as to whether the expenditure requested for the stated purposes will be a good investment or whether it can be made a good investment by expansion into a cooperative project or by other modification.

With respect to the kinds of conclusions that might be drawn from the work if it can be carried out successfully, there seem to be three possibilities:

(1) There may be a positive correlation between the degree of coronary arteriosclerosis and the amount of smoking.
(2) There may be a negative correlation, or
(3) There may be no correlation.

If there should be a positive correlation, the general situation would be much as it is already. That is, we would not really know whether the smoking caused the greater degree of disease by speeding up the process, or whether the association was indirect through other intermediate factors and not actually causal. Those who are eager to indict tobacco would undoubtedly seize upon any such result and employ it as another evidence that tobacco use is damaging to health, whether this conclusion was justified or not. Of course, if a sufficient number of other life habits could be studied simultaneously there would be a better chance of getting some real perspective.

If there should be a negative correlation, the degree of conclusiveness would be similar. We would not really be justified in concluding that tobacco use slows down the atherosclerotic process although this would be one possible hypothesis.

If there should be no correlation, the results would be more conclusive, since if the precision of the data were sufficient to make it certain that there was a real lack of correlation and not merely a methodological indeterminacy, we could then conclude that tobacco use actually does not accelerate atherosclerosis of the coronary arteries.

If my reasoning is correct, the study appears as a kind of gamble in which "heads we win but tails we do not lose for sure" (i.e., scientifically though we might in the newspaper headlines and even at the hands of "study groups").

1003546866

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Is there any basis for guessing what will be found? In a somewhat similar study, Spain found no correlation between smoking and the amount of disease in the coronary arteries.

At our conference, Thomas Dawber reported that the Framingham study found no correlation between smoking and the incidence of angina pectoris. They have done relatively little anatomic or pathological work. However, if one assumes that angina is a chronic manifestation of generalized atherosclerosis of the coronary arteries, the observation might suggest that probably no correlation would be found between smoking and the pathological condition found on autopsy. The Framingham group did find a positive correlation between smoking and myocardial infarction. The two observations can be reconciled by assuming that smoking might, directly or indirectly, contribute to the precipitation of an acute episode when the underlying conditions are conducive. This is a hypothetical concept, and I do not know of any mechanisms that have been elucidated or even proposed unless the reported but unsubstantiated effect of smoking on clotting time or hematocrit is considered to be such.

To determine whether smoking does or does not affect the rate of atherosclerosis by direct or indirect means would seem to be one of our most important mainstream problems. If we cannot get elucidation by study of human material, the chief alternative in sight is animal experiment. Our efforts along these lines with Dr. Wenzel were not very rewarding.

However, in a recent conference with Dr. Fredrick Stare, he expressed the opinion that animal experiments could be made meaningful. He thinks that dietary methods for producing atherosclerosis in animals are now sufficiently standardized and reproducible that studies could be designed to superimpose tobacco smoke inhalation upon a basic atherosclerotogenic regimen in half a dozen different species, including primates, and determine the effects of this added factor on the score.

The problem may be important enough to justify both human and animal studies. I plan to pursue discussions further with Stare and Ira Gore on the animal work.

Meanwhile we must determine how far we want to gamble on McGill's approach.

R.C.H.

RCH:ck

1003546867

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY & CHEMISTRY

NO. 632
cf. #174
Activated: 2/1/58
Renewed: Annually
on 2/1 - 1959-'64
Inclusive

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE
Dr. Reimann, Chm.
Dr. Bing
Dr. Cattell
Dr. Sommers

Application For Research Grant

Date: August 22, 1967

1. Name of Investigator(s): (include Title and Degrees)

Jack P. Strong, M. D. - Myra L. Richards, A. M.

2. Institution & Address:

Louisiana State University, Baton Rouge, La.

3. Short Title of Project:

Relationship of Smoking and Other Environmental Factors to Atherosclerotic Lesions

4. Proposed Starting Date: February 1, 1968

5. Anticipated Duration of this Specific Study: 3 years

6. Brief Descripton of Objectives or Specific Aims:

With reference to studies already in progress and to others being considered in the Department of Pathology at the Louisiana State University Medical Center, we wish to request reinstatement of support for continuation of our present autopsy study of the relationship of smoking and other environmental factors to atherosclerotic lesions. Support toward this retrospective study of a cumulative sample of autopsied male deaths in New Orleans was provided by the Tobacco Industry Research Committee throughout the major developmental and testing phases begining February 1, 1958, and on through the first 3 years of the definitive phase. The last grant year ended on January31, 1966, with an extension to July 31, 1966, for the use of unexpended funds.

The purpose (and plan) of our study continues to be to investigate selected environmental factors, including lifetime history of cigarette usage, occupation and occupational physical activity (last 5 years), dietary practices, habitual level of salt intake, and educational level attained, in relation to various measurements of type and extent of atherosclerotic lesions in the aorta and coronary arteries.

1003546868

7. Give a Brief Statement of your Working Hypothesis: For human males, holding age, race, and cause of death classification constant, amount of cigarette smoking is positively associated with atherosclerotic involment of both the aorta and the coronary arteries.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

The sample of cases to be investigated is comprised of white and Negro resident males, aged 20 through 64 years at death, who die in Orleans Parish and who are autopsied at the Charity Hospital of Louisiana at New Orleans or the laboratories of the Parish Coroner. Reliable visual and radiographic methods, previously developed in our laboratories in connection with the International Atherosclerosis Project, for quantitation of various aspects of lesion development are applied by the pathologists to the specimens which are dissected at autopsy by our technicians, processed for preservation, and stored for reevaluation. The specimens are blind-coded, and prior to the evaluation, the pathologists have no information pertaining to the identification of the subject or to the clinical or autopsy data.

The collection and preparation of the pathological data and that of the environmental data are two distinct and separate operations. Research associate interviewers, adequately trained and experienced in social work, obtain the data by interview of surviving family members or other close associates, and prepare the basic data for transfer to IBM punch cards by the Department of Biometry, LSUSM, where it is programmed for computer analysis. Prior to the analysis stage of the study, the interviewers have no knowledge of the artery findings. (see attached sheet for continuation)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

There is a large research team in the Department of Pathology engaged in long term research on atherosclerosis. The head of the department (principal investigator), co-investigator, and chief of the biophysics section are directly concerned with providing professional supervision, interviewing, (see attached sheet for continuation)

10. Additional Requirements:

NONE

11. Biographical sketches of all principal and professional personnel (append)

Jack P. Strong, M. D. - Myra L. Richards, A. M.

12. List of publications: (Five most recent as pertinent) (append)

See attached sheet.

100354869

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Recognizing that a sample of necropsied subjects is a selected group and therefore not representative of the living population, it is necessary that comparative study of the values derived by measurement be made within appropriate race-age groups, subdivided further according to major cause-of-death classification. In this connection, preliminary tabulations were made in late 1966 of the first 645 cases completed, and in Table I (attached) is presented the race-age distribution of the sample according to the classification of "basal", without evidence of Coronary Heart Disease (CHD), and "non-basal", with evidence of CHD.

The sample is 54% Negro and 46% white. It is of interest that 208 (60%) of the 347 Negro cases fall into the basal group, while the 298 white cases break about evenly between basal and non-basal, 51% and 49%, respectively. It follows that, since 45% of all Negro cases fall in the younger age groups (20 through 44 years), a large proportion of the Negro cases would be basal, --that is, free of evidence of CHD or disease thought to be related to CHD or to smoking (hypertension, stroke, diabetes, lung cancer, emphysema).

We are preparing for an interim analysis of the present total of 756 cases in which both the pathological and the environmental data have been transferred to punch cards. This interim sample represents approximately the half-way point in reaching our revised goal of a 1,500-case sample. (Revision of the original goal of 500 cases was necessary in order to amass a greater number and a more nearly equal distribution of the cases among the sub-groups, thereby permitting more meaningful analyses.)

Environmental histories have been obtained at interview of qualified family members or other close associates in another 200 cases. These cases now await coding and the visual and radiographic measurements of the lesions by the pathologists and by the staff of the Biophysics Section, Department of Pathology. Another 40-odd new cases were assigned for interview in April, and upon completion of these and others awaiting assignment, the total cumulated sample should exceed 1,000 cases by September first. This cumulation of cases will represent the obtainable environmental data on all white and Negro male necropsied deaths, beginning January 1, 1963, whose artery specimens were collected by our technicians at necropsy and who have met the criteria for the study sample as defined in our Standard Operating Protocol. (The autopsies of the 70 male cases of the pilot study, now included in the study sample, were performed between June 1, 1961, and May 31, 1962.)

At our present rate of completing the cases, the collection of the last 500 will require approximately 2 years, and the analysis of the data for the total 1500-case sample, one additional year.

Continuation to question 9, second page

or obtaining and evaluating anatomic material for this specific project. A medical research technologist and typist-clerk are engaged in the project and there is a position open for a social worker interviewer. Ample autopsy material from Charity Hospital of Louisiana at New Orleans and the Office of the Coroner, Orleans Parish, is available. The Department of Biometry LSU School of Medicine has supervised the development and testing of the methods of collecting information on smoking habits and is collaborating in analyzing the data.

10035468870

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Table 1. Distribution of the Cumulated Sample of 645 Cases (N) for Preliminary Analysis, of the Basal Group of 360 Cases, and of the Non-Basal Group (the Remainder) of 285 Cases, by Race and by Age.

AGE	Number of Cases in Sample (N)	WHITE			NEGRO		
		All (white)	Basal Group*	Non-Basal** Group (the remainder)	All (Negro)	Basal Group*	Non-Basal** Group (the remainder)
20-24	38	14	13	1	24	21	3
25-34	89	29	27	2	60	52	8
35-44	122	51	31	20	71	47	24
45-54	191	93	43	50	98	46	52
55-64	205	111	38	73	94	42	52
TOTAL	645	298 (46% of sample)	152 (51% of white)	146 (49% of white)	347 (54% of sample)	208 (60% of Negro)	139 (40% of Negro)

*"Basal" = those subjects without evidence of CHD or disease related to CHD or to smoking

** "Non-Basal" = those subjects with " " " " " " " " " " "

October 3, 1966

10035468?1

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Myra L. Richards, A.M.	100	
Technical		
Mariz Longoria, Typist-Clerk III	50	
Junius Solomon, Medical Research Technologist.	50	
Sub-Total		
B. Consumable Supplies (list by categories)		
Computer time	500	
Interview forms and supplies	1000	
		1,500
Sub-Total		
C. Other Expenses (itemize)		
Travel to professional meetings; one trip to England(London School of Hygiene)for consultation on analyses and results(May not be utilized until following year).		500 1,000
Sub-Total		
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)	2,675	17,841
Total		20,516

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	1,500	Travel 500		2,586	19,826
Year 3	R	1,500	Travel 500		2,646	20,283

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature] Director of Project Telephone

Signature [signature] Business Officer of the Institution Telephone

10035482

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Natural and Experimental Atherosclerosis	U. S. P. H. S.	$260, 000	7 years
	Organized Research Budget supporting Atherosclerosis research.	L. S. U. School of Medicine	30, 000	1 year
Pending				

10035468?3

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Jack Perry Strong, M. D.

R

Marital Status: R

Secondary Education: Phillips High School - 1941 - 1945, Birmingham, Alabama

College: B. S. University of Alabama, 1948

M. D. Louisiana State University School of Medicine, 1951

Honors:

Phi Beta Kappa - 1947
Alpha Omega Alpha - 1950
Phi Kappa Phi - 1967

Academic and Professional Appointments:

Internship (rotating), Jefferson Hillman Hospital, Birmingham, Alabama, 1951 - 1952
Assistant, Department of Pathology, Louisiana State University School of Medicine, 1952 - 1953
Medical Officer and Pathologist, U.S.A.F., 1953 - 1955
Consultant in Pathology, Southwest Foundation for Research and Education, 1954 - 1955
Instructor in Pathology, Louisiana State University School of Medicine, 1955 - 1957
Assistant Professor of Pathology, Louisiana State University School of Medicine, 1957 - 1960
Associate Professor of Pathology, Louisiana State University School of Medicine, 1960 - 1964
Assigned to Professor J. N. Morris, Social Medicine Research Unit of the Medical Research Council, London, England September 1962 - September 1963
Combined Course in Medical Statistics by Professors P. Armitage and D. D. Reid, London School of Hygiene and Tropical Medicine, January - April, 1963
Professor of Pathology, Louisiana State University School of Medicine, 1964 to date
Head, Department of Pathology, Louisiana State University School of Medicine, 1966 to date

10035468?4

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Hospital Appointments:

Assisting Visiting Pathologist, Charity Hospital of Louisiana at New Orleans, 1952 - 1953; 1955 - 1958

Visiting Pathologist, Charity Hospital of Louisiana at New Orleans, 1958 to 1966

Senior Visiting Pathologist and Pathologist-in-Chief, LSU Division, 1966 to date

Consultant Appointments:

Louisiana Heart Association Research Committee, 1960 to date
Pathology A Study Section, United States Public Health Service, 1965 to date
World Health Organization Atherosclerosis Project, 1962-1963

Fellowships:

Senior Research Fellow, United States Public Health Service, 1957 - 1962
Research Career Development Award, United States Public Health Service, 1962-1964

Professional Organizations:

American Association of Pathologists and Bacteriologists; Assistant Secretary, 1959-1962
American Society for Experimental Pathology
International Academy of Pathology
American Society of Clinical Pathologists; Councilor for Louisiana, 1966
American Society for the Study of Arteriosclerosis
Diplomate of American Board of Pathology - Pathologic Anatomy, 1957; Clinical Pathology, 1958
College of American Pathologists

Research Interests:

Epidemiology, geographic pathology and pathogenesis of atherosclerosis; atherosclerosis in primates

Current Grants:

Natural and Experimental Atherosclerosis (HE-08974)
Pathology Training (GM-01202)
Frost Foundation

Community Activities:

President, Bissonet Elementary Parent Teachers Association, 1961
Vice President, Jefferson Committee for Better Schools, 1964
Member of Official Board, Munholland Methodist Church, 1965

10035468875

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

[redacted]

Curriculum Vitae Myra L. Richards July 1967

Place of birth: REDACTED

Marital status: REDACTED

Education

Secondary: Liberty High School

College: [redacted]

Graduate: Tulane University:

School of Social Work* Specialty, Medical-Social } 1930-1932...Certificate
Completion of the 2-year curriculum
Graduate School.....1930-1932...A.M.degree

Experience

Social Service Dept., Charity Hospital	- (see page 2 for positions held in the Dept.)	1932-1942
La. State Univ. School of Medicine	- Field Supervisor of 6 senior medical students in "Social Aspects of Medicine"	1933-1934
Public Health Dept., Night V-D Clinic	- Medical-Social Worker.....	1934-1935 (approx. 5 mos.)
Social Service Dept., Charity Hospital	- Medical-Social Worker... part-time Eye clinics and wards.	1955 (Sept.-Nov.)
Social Research, Inc. (of Chicago)	- Survey interviewer... part time N.O.Dir., Dr.Rob't.Stone, Tulane: (1)"alcoholic beverages"; (2) "attitudes toward the dr ft (parents of draft-age boys, prior to induction).	1957, 1958
La. State University Medical Center Dept. of Pathology	- Research Assistant(Clinical).. part-time...	1956-1958
	- Research Associate(Clinical).. part-time...	1958-1960
	- Associate (Research).....	1960-1966
	- Instructor.....	1966 to date

10035468876

*Graduate School:

Tuition Award (library).....(1 yr.)
Emerg. Relief Admin...certification interviewer.....1931 (3 months)

Social Service Dept. - Medical-Social Worker.....1931-32 (half-day)
Charity Hospital

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Social Service Dept., Charity Hospital, New Orleans, La., Positions Held:

July, 1934 - Medical-Social Case Worker --all white clinics.

Dec.1935-Oct.1937 (approx.) - Formulated operational policies and procedures for a joint W.P.A. - Charity Hospital medical certification project, (staffed by WPA physician and registered nurse) for screening applicants; served as liaison worker in the Charity clinics for applicants who required further examination and evaluation for work.

July, 1936 - Medical-Social Case Worker -- 9 general and special white clinics and wards.

Oct., 1938 - Organized and supervised the Dept's. Intake Service; supervised clerical staff of 15 and an N.Y.A. clerical project.

Jan., 1940 - Set up and supervised the first program of medical-social services in Charity Hospital Admitting and Accident Rooms.

Feb., 1941 - Designed and supervised the hospital's first program of establishing eligibility by standardized interview procedure; trained the interviewers.

Case Supervisor of 7 medical-social workers in clinics and Admitting Room.

June-Dec. 1941 - Director, Social Service Dept. (acting)---professional staff of 35 medical-social workers and clerical staff of 16. (Unable to accept permanent appointment as husband was to be commissioned in War II).

Dec.1941-July 1942 - Supervisor: Charity Hospital Admissions Unit interviewers and 7 medical-social workers in clinics and Adm. Dept. (Resigned to join husband at air base --War II).

Sept.-Nov., 1955 - (See preceding page).

Professional Organizations

REDACTED REDACTED REDACTED

Publications

Co-author - "Relationship Between Cigarette Smoking Habits and Coronary Atherosclerosis in Autopsied Males." Circulation, Suppl. No. III, October 1966, Vol. XXXIV, No. 4, p. 31. Abstract.

10035468777

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS

Strong, J. P., and H. C. McGill, Jr. 1962. The natural history of coronary atherosclerosis. Amer. J. Path. 40(1):37-49

Strong, J. P., and H. C. McGill, Jr. 1963. The natural history of aortic atherosclerosis: relationship to race, sex, and coronary lesions in New Orleans. Exp. Molec. Path., Suppl. 1:15-27.

Strong, J. P., and D. A. Eggen. 1965. Atherosclerotic lesions, p. 57 to 72. In The Milbank Memorial Fund Quarterly, Comparability in international epidemiology. Milbank Memorial Fund, New York.

Eggen, D. A., J. P. Strong, and H. C. McGill, Jr. 1965. Coronary Calcification: relationship to clinically significant coronary lesions and race, sex, and topographic distribution. Circulation 32:948-955.

Strong, J. P., H. C. McGill, Jr., M. L. Richards, and D. A. Eggen. 1966. Relationship between cigarette smoking habits and coronary atherosclerosis in autopsied males. Circulation 34 (Suppl. 3):31. (Abstr.)

1003546878

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

COUNCIL ON ARTERIOSCLEROSIS - AMERICAN SOCIETY FOR THE STUDY OF ARTERISOCLERO.

Committee:
Dr. Reimann
Dr. Ping
Dr. Cottell

Return to:
AMERICAN HEART ASSOCIATION, 44 East 23rd Street, New York, N.Y., 10010

#174R7

Activated: 2/1/56
Renewed annually on 2/1

DO NOT FILL IN! 59 - '6 Incl.

Abstract of paper to be considered for presentation at the
20th Annual Meeting, October 19-20, 1966
New York, New York

DEADLINE for submission: postmarked MAY 16, 1966

TYPE ALL INFORMATION! DO NOT USE INK FOR CORRECTIONS! SUBMIT IN DUPLICATE!

Number A-

Rec'd

RELATIONSHIP BETWEEN CIGARETTE SMOKING HABITS AND CORONARY ATHEROSCLEROSIS IN AUTOPSIED MALES

1. Jack P. Strong, M.D. New Orleans, Louisiana
2. Henry C. McGill, Jr., M.D. New Orleans, Louisiana
3. Myra L. Richards, M.A. New Orleans, Louisiana
4. Douglas A. Eggen, Ph.D. New Orleans, Louisiana

In order to test the association of cigarette smoking with coronary atherosclerosis, we collected coronary arteries from 645 autopsied males, 20-64 years of age. Interviewers obtained estimates of cigarette smoking habits of these deceased persons from surviving relatives. The interviewers used schedules that had been tested for validity and reliability in pairs of living persons from the same household. Independently, we estimated visually the extent of each type of atherosclerotic lesion and measured the extent of calcification and mean coronary wall thickness by optical-electronic scanning of radiographs. For the principal analyses we excluded patients having diseases thought to be associated with smoking (emphysema, lung cancer, etc.) or with coronary heart disease (myocardial infarction, hypertension, diabetes, stroke, etc.). The mean percent of the coronary intimal surface occupied by raised atherosclerotic lesions (fibrous plaques, complicated lesions, or calcified lesions) was approximately twice as great in heavy smokers (≥ 25 cigarettes per day) and about one-third greater in light smokers (< 25 cigarettes per day) than in non-smokers. Calcified lesions and mean coronary wall thickness measured radiographically were, on the average, highest in heavy smokers and lowest in non-smokers. Differences among these smoking categories were generally greatest at younger ages. Occupational physical activity and educational level achieved could not account for observed differences in extent of lesions.

Supported by NHI grant HE 08977 and by a grant from the Tobacco Research Council.
Council on Tobacco Research-U.S.

1003546879

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

October 4, 1966

Jack P. Strong, M.D.
Professor of Pathology
School of Medicine
Louisiana State University
1542 Tulane Avenue
New Orleans, Louisiana 70112

Dear Doctor Strong:

Mr. Hoyt has turned over to me the abstract you prepared for the American Heart Association meeting in New York on October 20th. It has been read with great interest. Indeed we have been awaiting the results of this long-term study rather impatiently in the expectation that they would considerably influence the future direction and emphasis in our research program. This promises to be the case.

If you had found a definite lack of correlation between cigarette smoking and the degree of atherosclerosis, some rather definite conclusions might have been warranted. We could have assumed, rather definitely, that neither chronic nicotine absorption at customary levels, nor the absorption of any other cigarette smoke ingredient significantly influences the atherosclerotic processes, and could virtually have written this off as a possibility. We could then have focussed our attention chiefly upon the still-obscure mechanisms that precipitate acute clinical events such as thrombosis and/or infarction in subjects who have developed a predisposing degree of sclerosis. An informal conference on this latter subject was held in our offices on June 10, 1966, attended by about a dozen specialists. They seemed to agree that though atherosclerosis tends in general to predispose to infarction, yet the latter frequently occurs where there is relatively little atherosclerosis and often fails to occur where such lesions are very advanced. An effort was made to devise some experimental approaches to this problem, in the expectation that our main future interest in cardiovascular disease would lie in this area.

We had rather expected that you would find such a lack of correlation. The reasons for this expectation were as follows:

1. Our own animal studies involving superposition of chronic nicotine dosage upon an atherogenic diet showed no consistent differences between the animals receiving the alkaloid and those on the diet alone.

2. The similar post-mortem pathology studies of Wilens and Plair and of Spain, which showed little association.

3. The Framingham-Albany studies showing that smokers develop angina pectoris no oftener or earlier than non-smokers.

1003546880

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Jack P. Strong, M.D.

4. The fact that cigar and pipe smokers experience little greater mortality from ischemic heart diseases than non-smokers although they probably absorb as much nicotine as cigarette smokers. (This remains to be demonstrated definitely).

My reasoning throughout the course of your study has been that the demonstration of a non-correlation would permit definite scientific conclusions. On the other hand, the finding of a positive correlation would leave us, from the standpoint of logic, very nearly where we were before. That is, the possibility that nicotine (or some other smoke ingredient) may contribute to the atherosclerotic process would remain open, but the existence of a correlation would not be sufficient to demonstrate that nicotine or other ingredient actually does so. The reason, of course, is that the subjects were not randomized before the imposition of nicotine dosage (or smoking) as would be the case in an animal experiment, but were self-selected. If smoking is a response to constitutional factors of temperament, personality, ability to cope with stress and anxiety etc. and is therefore associated with a whole "style of life" that is different from that of non-smokers, as many evidences seem to indicate, then these factors or life habits (exercize, sleep, use of aspirin, coffee drinking, alcohol consumption, diet etc.) might themselves be the causes of the differences in degree of arterial atherosclerosis and only be reflected in the smoking practises.

Your findings suggest that we may have to undertake still further controlled animal experiments, hopefully using better models and design than the previous ones, to isolate and evaluate any specific nicotine effect that may exist. They also suggest that we may need to extend further our studies of personality and "style of life" in smokers as compared to non-smokers. Still further, they suggest that we must continue and intensify our efforts to perfect biochemical methods of comparing the actual nicotine absorption by pipe, cigar and cigarette smokers under their habitual conditions of tobacco use.

I am afraid that when this paper is presented publicly, we may be called upon for comment, though we generally dislike to do so in the public press. If compelled to do so, the kinds of comments we would be inclined to make are implied in those outlined above. It would be quite unfortunate, I think, if there appeared to be any disparity between your views and ours on the interpretation or implications of these results. That there should be any actual disparity seems to me very unlikely, but reporters sometimes like to create the appearance of a controversy. This could probably be avoided if you see the implications as we see them and disarm the press by including in your presentation a caution against overinterpretation. Then, if we were forced to comment, we could simply emphasize our agreement with you.

1003546881

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Jack P. Strong, M.D.

If you feel that this matter needs discussion I can probably arrange to stop off in New Orleans on my forthcoming trip to Texas.

Very sincerely yours,

RCH:tih
enclosure

Robert C. Hockett, Ph.D.
Associate Scientific Director

P.S. If full copies of the various papers you are presenting at the meeting could be made available to us in advance, it would be very helpful.

100354682

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LOUISIANA STATE UNIVERSITY MEDICAL CENTER
1542 TULANE AVENUE · NEW ORLEANS · LOUISIANA · 70112
School of Medicine
DEPARTMENT OF PATHOLOGY

October 13, 1966

Dr. Robert C. Hockett
The Council for Tobacco Research
633 Third Avenue
New York, New York 10017

Dear Dr. Hockett:

I appreciate the comments included in your letter to me concerning the abstract on Smoking Habits and Atherosclerotic Lesions. Your comment concerning future experiments was particularly apropos to our situation since we have been carrying on pilot studies for using primates to study the effect of smoking on atherosclerosis. We are now at the stage where we need additional grant support for animals, cages, and some personnel for more expanded studies. I would like to make application to The Council for Tobacco Research to support some of this work and would appreciate it if you would send me the necessary application forms.

Sent 10/21/66 →

Many thanks for your support in the past.

Sincerely,

Jack P. Strong, M. D.
Professor and Head

JPS:lml

1003546883

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A.

October 26, 1966

MEMORANDUM

TO: CCL

FROM: JMB

SUBJECT: The 39th Scientific Session of the American Heart Association, New York City, October 21-28, 1966. Also the American Society for the Study of Arteriosclerosis and the Council on Arteriosclerosis of the American Heart Association, October 19-20, 1966.

The paper by CTR Grantee Jack Strong, "Relationship between Cigarette Smoking Habits and Coronary Atherosclerosis in Autopsied Males," was the only story of the meeting picked up by The New York Times on Friday, the 21st. This paper pointed out the 'association' of cigarette smoking and higher incidence of atherosclerosis in the heavy smoker. Dr. Strong concluded that smoking per se, was not a major cause of atherosclerosis but that smoking, plus some unknown factor could be, or well may be, the cause of increased incidence of atherosclerosis in the smoker. Dr. Engelberg asked if the number of patients studied were statistically significant. Strong's reply: "The numbers are fairly small and are questionably significant." The report in The New York Times inferred that Strong had stated a direct causal relationship. No mention was made of the influence of nutrition in this study. Myra Richards reported that they were still working over this material and indicated that there appeared to be a high correlation between poor nutrition and atherosclerosis.

Dr. H. McGill reporting on the "Geographical Pathology of Atherosclerosis," an international study of 17 countries still in progress. -- It appears that these specimens are collected from the lowest socio-economic group of the participating countries. A written report is due in the literature in a few months. Dr. McGill has been appointed to the staff of the new medical school under construction in San Antonio, Texas.

A paper on "Coronary Artery Disease in Migratory Steelhead Trout" was read by Dr. Van Citters of Seattle. The pathology consists of intraluminal proliferations containing intimal tissue and fragmented internal elastic membranes. These changes are thought to be due to hyperadrenocorticism as an accompanying part of sexual maturation. This change in the salmon results in death of the fish after spawning. The steelhead trout has the same changes occur while in fresh water. However, the steelhead may return to salt water once or twice during the life span - the arterial changes completely reverse after returning to salt water. There is no sex difference. The salmon has no lipid or fat of any kind in the blood stream. Major interest is the degeneration of the arterial wall resulting from accelerated adrencortical activity.

Dr. Siperstein of Dallas, presented new material on the thickening of the basement membrane of the capillary. While all capillaries show this change, the specimens for the study were all taken by needle biopsy from the gastrocnemius muscle. This change was noted in all diabetics and pre-diabetics. In addition to the usefulness of this technique in diagnosis, this paper fit into a number of other papers involving variants in carbohydrate metabolism as a basic precursor to degenerative vascular disease. If this capillary change is uniformly demonstrated, then perhaps the vaso vasora of the great vessels are the forerunner of degenerative disease of the aorta.

J. Morrison Brady, M.D.

1003546884

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

August 31, 1965

Henry C. McGill, Jr., M.D.
Acting Head
Department of Pathology
Louisiana State University School
of Medicine
1542 Tulane Avenue
New Orleans 12, Louisiana

Dear Doctor McGill:

We have recently noted some publicity in the papers concerning a project to teach baboons to smoke cigarettes. We would be interested in learning a little more about these studies - whether they are entirely preliminary or exploratory or part of an extensive plan.

Our own smoke inhalation studies so far have involved only mice, rats, hamsters and dogs. The exposure of these small animals by the inhalation route involves many problems. The smoke has to be generated by mechanical means, keeping the conditions of combustion close to those used by humans in normal smoking. The smoke has to be diluted with the proper proportion of air and delivered to the lung surfaces at the right age, in seconds or fractions thereof, following generation at the burning zone. These problems might be simplified in the case of an animal capable of smoking in the manner of humans, but of course other problems arise such as number of animals, housing, costs etc.

A special problem with the smaller animals is that the smoke really ought not to be allowed to pass through the nasal passages which may filter out a substantial proportion of the particulate matter so that it does not reach the lung at all. Most of the work with these species so far has involved nasal inhalation.

Still another important problem that includes both man and baboon is to devise a method of measuring the actual exposure of lung surfaces to total smoke. The number of cigarettes lighted up in a day by a human smoker (as deduced from questionnaires) is not a very good measure of lung exposure because of variations in the numbers of puffs actually taken and the depth of inhalation, if any. For this reason, we have been endeavoring to develop a physiological or biochemical method of monitoring or estimating the actual lung exposure.

1003546885

The most promising method so far is the measurement of blood carbon monoxide. This gas is present in a small but fairly constant concentration in cigarette smoke. The evidence is that it is little absorbed through the tissues of the oral area, but is avidly absorbed through the alveolar membranes. Since humans can puff by the stop-watch, and inhale or not as directed, we have been able to start at standardizing the blood-CO response to regulated smoking procedures. Although carbon monoxide is eventually eluted from

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

the blood again, this process, unlike the metabolism of nicotine, is slow enough so that analytical work can be carried out comfortably without undue danger of confusion in the results attributable to changes during the time between exposure and sampling.

After sufficient standardization, we hope that we can use blood sampling more or less routinely as a method of checking subject questionnaire data on numbers of cigarettes smoked (or rather burned) per day. Discrepancies between numbers lighted and amounts of smoke actually inhaled may be quite instructive with respect to nervous habits, for example. A man who lights a large number of cigarettes but takes few real puffs may be quite a different kind of person from one who lights relatively few but really smokes them. Inhalers may be quite different from non-inhalers and we doubt whether subjects can ordinarily judge very well whether they inhale or not, all aside from any emotional factors that may prejudice what they say they do.

I think that in humans carbon monoxide absorption may be a fairly good indicator of lung surface exposure to whole smoke, including particulate matter. While there must be some loss of particulates in the mouth, throat and larynx as the aerosol passes through to the lung, this should be reasonably nearly constant in humans. It may not be the same for rats, mice and hamsters if these breathe through the nose. The fraction of the original particulate matter which reaches the lung may be considerably lower in these animals, if they breathe the smoke in through the nasal passages, as they generally have done in the experiments carried out in the past. The fraction might well be fairly constant in this case also, but constant at a lower level in relation to the carbon monoxide reaching the alveolar. The same blood analysis might be used with the small animals, if once a method of standardization could be carried out by use of some marker present only in the particulate matter. Nicotine is probably not a good one because of its rapid absorption and metabolism, though Harris and Negroni have used determinations of nicotine in mouse lungs as a qualitative indication that some particulate matter is reaching this organ.

In any case, if extensive experiments are to be done with baboons, the question of the extent of exposure of the lung tissues is likely to arise again if any effort to observe dose-response relationships is to be made, as it certainly must sooner or later.

A progress report on one of the human studies is being enclosed for your information. It should, of course, be regarded as confidential and not cited as such until published.

With kind regards and all best wishes,

Sincerely yours,

Robert C. Hockett, Ph.D.
Associate Scientific Director

1003546886

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LOUISIANA STATE UNIVERSITY MEDICAL CENTER
1542 TULANE AVENUE · NEW ORLEANS · LOUISIANA · 70112
School of Medicine
DEPARTMENT OF PATHOLOGY

September 9, 1965

Dr. Robert C. Hockett
Associate Scientific Director
The Council for Tobacco Research-U.S.A.
633 Third Avenue
New York, New York 10017

Dear Dr. Hockett:

Thank you for your informative letter of August 31 and the enclosed reprints. Publicity on this project, which started with a report in a local paper by one of the feature writers who has followed our work here quite closely, went a little farther than we had expected at this stage.

The project is entirely preliminary and is an attempt to eliminate some of the objections that you describe in your letter to the experimental work with smaller laboratory animals. In addition, it is our hope that if other animals can be taught to smoke cigarettes, we will be able to assign baboons randomly to the experimental group.

So far all efforts have been directed toward developing procedures for teaching the animals and we have carried out no monitoring experiments to test exposure of the lung surface to whole smoke. At any rate, we will certainly be able to eliminate the passage of smoke through the nasal passages since the animals smoke with the mouth and lips just as does man.

Any further information that you develop or that you come across relating to how one can determine actual exposure of the lung surface to smoke will be greatly appreciated.

Sincerely,

Henry C. McGill, Jr., M.D.
Professor and Chairman
Department of Pathology

HCM:lml
cc Dr. Jack P. Strong

1003546887

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

December 5, 1966

Jack P. Strong, M.D.
Professor and Head
Department of Pathology
Louisiana State University Medical Center
1542 Tulane Avenue
New Orleans, Louisiana 70112

Dear Doctor Strong:

In accordance with your request, we sent you application forms on October 21st, and assume that we would be receiving a proposal for an animal study on the effect of smoking and/or nicotine on atherosclerosis. I should point out that we are revising our forms and that a new modified version may be available in a few weeks. We will send you copies of these, and they may reach you in time for use in outlining your proposed investigation.

We have seen advance references to the work that you have been doing in collaboration with the Southwest Research Institute on smoking by baboons. The purpose of using baboons in cardiovascular studies, as I understand it, is that these animals develop lesions that are closely analogous to the human disease. Also, since they are omnivorous, it is said that they can be fed almost any kind of experimental diet that may be of interest in relation to cardiovascular disease. On the other hand, the cost of maintaining and studying baboons rather frightens me when I consider what must be involved. Their normal life span must be quite lengthy so that long periods of observation would be necessary. Another reason for the selection of baboons, according to some reports, is that they can be taught to smoke cigarettes as humans smoke them. This fact, if it is true, suggests that their use in behavioral studies might be quite interesting. On the other hand, one great problem, even in human studies of smoking, is the difficulty of relating actual smoke dosage to any biological or hypothetical consequence of smoking.

We have been conducting smoke inhalation experiments with small animals for about ten years. The original methods were pretty crude but, little by little, the techniques have been improved so that now we feel that we know how to carry out smoke inhalation exposures under conditions closely resembling those encountered in man, with objective measurements of actual exposure to both the particulate and gas phases of the smoke. We plan to extend these studies in an effort to get good dose response measurements in terms of pulmonary pathology.

In this field there are great advantages in the use of mice since well-standardized inbred strains are available, and relatively large numbers of animals can be used for lifetime experiments. The problem of a suitable animal model for studies of atherosclerosis appears to be somewhat difficult. Mice may

1003546888

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

not be very satisfactory for such work. We sponsored a few studies several years ago in which rabbits and hens, fed high cholesterol diets, were used as the model. This technique was not altogether satisfactory we thought, and we have hoped that a better model might now be available for a repetition of this kind of work.

We have been told for several years that a colony of small primates was to be set up in Boston in connection with the Harvard School of Public Health's Department of Nutrition. This has now been done and several varieties of small primates are being bred in captivity and standardized strains developed. Since dietary factors are regarded as having a relation to atherosclerosis, this colony has seemed to us to be a possibly promising locale for atherogenesis studies where standardized diets could be combined with either chronic nicotine dosage or actual smoke exposure. We have not yet entered into any actual negotiations with that group, and would certainly like to know more about the background work and pilot studies that have been carried out by you and your associates.

I think it would be a very good thing if we could get together in the very near future for a leisurely discussion of the whole problem and the various ways in which it might be approached. Around the turn of the year I am planning to make a trip to Houston and probably San Antonio on other matters. Perhaps we could also have a conference in the course of that trip, and if there is work on atherosclerosis in San Antonio that I should see I might make one visit serve several purposes.

I am enclosing copies of two publications that I happened to run into recently and which may be of some interest to you.

With kind regards,

Sincerely,

Robert C. Hockett, Ph.D.
Associate Scientific Director

RCH:ek

encls. 1. A. S. Milton. The effect of nicotine on blood glucose levels and plasma non-esterified fatty acid levels in the intact and adrenalectomized cat. Brit. J. Pharmacol., 26/1:256-263 (1966).
2. Robert E. Carroll. The relationship of cadmium in the air to cardiovascular disease death rates. J.A.M.A., 198/3:267-269, Oct. 17, 1966.

1003546889

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

LOUISIANA STATE UNIVERSITY MEDICAL CENTER
1542 TULANE AVENUE · NEW ORLEANS · LOUISIANA · 70112

DEPARTMENT OF PATHOLOGY

May 3, 1967

Robert C. Hockett, Ph.D.
Associate Scientific Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue
New York, N. Y. 10017

Dear Dr. Hockett:

Several months ago I spoke with you concerning a smoke substance "acetonitrile" which identifies smokers from non-smokers when isolated in the urine.

We are most anxious to learn more about this test as we would like to employ it in a study of deaths of 25-44 year old males in New Orleans. We plan to take smoking histories from members of the family who can be located but would like to have this objective type measure if possible. It would also be very helpful in those for whom we cannot find a respondent.

We would greatly appreciate any information which you are able to provide to us on this test.

Sincerely,

Jack P. Strong, M.D.
Professor and Head
Department of Pathology

JPS:tn

1003546890

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

May 9, 1967

Jack P. Strong, M.D.
Professor and Head
Department of Pathology
Louisiana State University Medical Center
1542 Tulane Avenue
New Orleans, Louisiana 70112

Dear Doctor Strong:

I have been planning to write to you ever since our visit last January, about the several items in my notes. Your letter now provides the stimulus to take action.

Acetonitrile

Most of what has been done on this is not yet published and we think that considerably more chemical work is still needed to validate the methodology and especially to solve the problems of kinetics in absorption and excretion. Studies on the subject were initiated at the Southwest Research Institute in San Antonio under government contracts and then continued under a grant from this Council. Before the work was completed, the man who was most concerned with it made a move. We have just received a "final" report but this leaves many loose ends. Under separate cover, we are sending you copies of the brief notes that have been published and excerpts from the reports we have. These are to be considered confidential and, as you will see, do not add up to a validated method ready for routine application. If you wish to get into this subject, it will require some constructive thinking and further research. The material is intended to save time and assist in research into applicability rather than as a description of a tested methodology.

Inhalation Studies

I think you have received a copy of our Annual Report for 1965-1966. On pages 11-13 you will find a report of our conference on techniques for exposure of experimental animals to cigarette smoke inhalation, including a statement of the conditions that we feel must be met to make such work really useful in describing dose-response relationships. Until there is some satisfactory method for controlling and estimating the actual lung surface exposures to the particulate and gas plus vapor phases of smoke, attempts to relate specific histopathological changes to dosage will remain rather vaguely qualitative. We are concentrating on these problems at present and have made some progress insofar as small animals are concerned. Small rodents, however, have not been used very much for studies of the cardiovascular system and, so far as I know, there is little background on the processes of arteriosclerosis in such animals.

1003546891

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Jack P. Strong, M.D.

We are just now undertaking an inhalation study with dogs to work out better methodology. We have not tackled primates along these lines. They will surely present a more difficult problem because of their manual dexterity.

For these reasons, I am sure our Board members feel that spontaneous smoking by baboons can, at present, contribute in a meaningful manner rather to study of psychological and motivational phenomena than to physiological, pathological or biochemical ones.

Atherosclerosis studies

The possibility of using primates, especially smaller ones, as a model for atherosclerosis studies seems more promising for the immediate future. If conditions can be established for producing lesions closely resembling the human ones, in a consistent and reproducible manner, it might be possible to superimpose chronic nicotine dosages (in water, food, pills or by injection) at several levels for observation of any differences induced by the alkaloid.

It would actually be much more interesting to superimpose whole smoke inhalation upon the atherogenic regimen, but the methodology for this seems not yet to be at hand. Even more interesting, perhaps, would be superimposed inhalation of the gas phase of smoke or of certain constituents, especially carbon monoxide (see Astrup). The last should be far easier experimentally since it would not involve the handling of a rapidly changing heterogeneous aerosol.

To make any such studies practical, we would need a basic atherogenic regime giving rather uniform effects so that we would not have to use unduly large numbers of animals.

Autopsy study

Among all the subjects we discussed, it seems most apropos to provide for continuation of your present autopsy study until a total of 1500 cases has been accumulated, and to continue analysis of data.

Arteries of young men, race relations.

This kind of thing undoubtedly ought to be done but I am uncertain as to how directly relevant to our purposes such studies will be considered by our Board.

Lung lipids

Since several studies on the effects of chronic influences on lung surfactant are now under way under our sponsorship or that of the A.M.A.E.R.F., I cannot easily judge whether our Board will feel that more is needed. It might depend on whether more promising methods or better experimental designs can be suggested.

10035468892

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Jack P. Strong, M.D.

We are enclosing a copy of the recent paper by Torbjorn Lundman. It presents very cogent evidence of the genetic influence in coronary heart disease as compared to environmental ones.

Meanwhile progress is being made in the study of how other habits and practises tend to "cluster" with cigarette smoking. Mr. Kurt Enslein, who is applying multivariate analysis to large bodies of descriptive data from epidemiological studies expresses this by saying that "smoking and drinking appear to be summarizing other and perhaps unmeasured variables". In terms of such variables, he is attempting to "describe" the smoker as compared to the non-smoker.

We hope that the combination of data from such more sophisticated epidemiological studies and experimental ones will produce some definite answers in time.

With kind regards,

Sincerely yours,

Robert C. Hockett, Ph.D.
Associate Scientific Director

P.S. We enclose three sets of our revised application form. The next logical target date will be the September meeting of our Scientific Advisory Board. The deadline is August first but earlier receipt is an advantage since it spreads out the Board's work load.

1003546893

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

NEW APPLICATIONS

1003546894

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546895

#611 - ROWLANDS

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 2, 1967

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Cattell and Dr. Bing.

FROM: Robert C. Hockett

SUBJECT: A new grant application from John R. Rowlands, Ph.D. - No. 611.

Enclosed herewith is a new project proposal with supporting material from John R. Rowlands, Ph.D. of Southwest Research Institute, San Antonio, Texas.

The preliminary investigations have shown an electron paramagnetic resonance (EPR) pattern believed to be produced by the interactions of free radicals and/or reactive diamagnetic molecules with a metal complex, presumably hemoglobin, present in the cells of rabbit lung tissues subjected to smoke. The exact nature of this interaction or bonding is not clear, but presumably entails covalent bonding to the sixth coordination site on the iron of hemoglobin - the same point at which oxygen is bound.

A study by Dr. John Waugh at M.I.T. has shown the EPR spectrum of interacted free radicals in the smoke condensate. He has not worked with lung tissue. He suggested the broad absorption may be due to a "two-electron triplet state" or to compounds and complexes of transition metals.

These studies are primarily definitive chemistry in approach. Further work will be necessary to define the interaction and suggest a means of separating the complex mixture undoubtedly involved.

The application has been under study for over a year and was distributed to the committee on March 29, 1967, but has not yet been considered by the S.A.B.

Robert C. Hockett
John H. Kreisher

1003546896

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CHEMISTRY

No. 611

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Cattell
Dr. Bing

Application For Research Grant

Date: May 27, 1966

Refer for critique from Industry Technical Committee.

1. Name of Investigator: John R. Rowlands

2. Title: Senior Research Scientist

3. Institution & Address: Southwest Research Institute
8500 Culebra Road
San Antonio, Texas 78206

4. Project or Subject: A detailed investigation of the nature of the reaction between biological materials and atmospheric contaminants using Electron Paramagnetic Resonances and optical spectroscopic techniques is proposed. Such a program could lead to the development of experimental methods for assessing the relative importance of various atmospheric contaminants as biological hazards.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

In recent years a great amount of statistical information has lead to the belief that there exists a close relationship between cigarette smoking and lung cancer. In addition, studies of this sort have also indicated a significantly higher incidence of lung cancer in areas of high atmospheric polution. It has so far proven to be very difficult to establish in an objective way the nature of the effects of air polution and smoking because of the lack of a convenient experimental method.

The advent of Electron Spin Resonance spectroscopy has provided the most powerful experimental technique that is currently available for the detection and identification of both free radicals and compounds containing paramagnetic metals. The technique has been used with great success in this last decade in such diverse areas as radiation biology, chemical kinetics and providing important experimental parameters for improving ligand field theory. Instead of discussing in detail here the experimental and theoretical aspects of electron paramagnetic resonance we have included as appendices two reprints which indicate in some detail the potential of the method.

1003546897

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

In the course of studies sponsored by NCI involving the chemical nature and properties of the constituents of tobacco smoke we have demonstrated that the reactive species found in the smoke interact quite dramatically with lung tissue to produce a complex electron paramagnetic resonance signal. The cigarettes were smoked directly into freshly excised rabbit lung. Details of our experimental procedure and results will be published shortly. The most interesting feature of the results is that the resonance signal we observe is not just that of a free radical signal; but consists of both a free radical signal and a resonance which may be attributed to at least one type of paramagnetic metal complex. No electron paramagnetic resonance signal was detected in control lungs under the same conditions used for the smoked lungs. The paramagnetic metal resonance signal does not exist in cigarette smoke itself and has obviously been produced by the reaction between cigarette smoke and lung tissue. We have not as yet identified the species giving rise to the signal but work is currently in progress in an attempt to make this identification. We have included as Figures 1, 2 and 3 the spin resonance signals of trapped cigarette smoke, a rabbit lung which has smoked six cigarettes and a rabbit lung which had smoked no cigarettes.

In a brief series of experiments we have also shown similar reactions of lung tissue with common atmospheric pollutant gases suggesting that a thorough investigation of the reaction between such contaminants and freshly excised lungs in addition to certain model biochemical systems such as metal-porphyrin complexes, has great promise in the study of the biological effect of atmospheric contaminants. It is suggested that the Council for Tobacco Research-U. S. A. will find in this experimental procedure a method which has the potential of allowing quantitative estimates of the biochemical (biological) effects of pollutants and further, of allowing a study of the combined biological effects of pollutants and tobacco smoke.

1003546898

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

6. Budget Plan:

See addenda for detailed breakdown for first year cost

a.	Salaries	_____
b.	Expendable Supplies	_____
c.	Other Expenses	_____
d.	Permanent Equipment	_____
e.	Overhead (15% of a, b, c)	_____
	Total	_____

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available: In addition to the senior staff listed on the cost estimate and the few small items of equipment requested in the budget all the remaining facilities and personnel are available and will be assigned as requested.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of support):

The proposed program is designed to be complimentary to the existent NCI program entitled "Physical & Chemical Properties of Free Radicals and Alkylating Agents in Tobacco Smoke," which deals explicitly with the effect of tobacco smoke alone. It is anticipated that in the latter phases of the proposed program efforts will be devoted to the study of the synergistic effects of tobacco smoke coupled with atmospheric pollutants. Together these two programs should provide an excellent experimental approach to the vexing program to the connection between the breathing of contaminants of one form or another and the onset of lung cancers.

In order to indicate the scope of our action in the approach of electron spin resonance to a variety of chemical and biochemical problems we have appended a partial listing of sponsored programs both completed and in progress.

Signature J R Rowland S
Director of Project

[signature]
Business Officer of the Institution

1003546899

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

COST ESTIMATE

First Year

A. Direct Labor Cost		
J. R Rowlands, Principle Investigator		$ 5,460
Research Scientist and Tech. Assistants		13,520
Total Salaries and Wages		$18,980
B. Material and Supplies		
Animals and animal supplies	500	
Miscellaneous chemicals and glassware	1500	2,000
C. Other Expenses		
Travel Expenses - Based on 2 Man Trips to New York		
Transportation	500	
Subsistence - Based on 5 Man Days	100	
Outside Consultant (Mr. Arthur Gross, Pico Laboratories) 10 days at $125/day	1250	
Photography	250	
Telephone Tolls and Telegraph	100	2,200
D. Surcharge		
110% Salaries		20,878
E. Special Equipment		
Miscellaneous optical components for spectroscopic equipment		2,000
Total Estimated Cost		$46,058

Signature J R Rowlands
Director of Project

1003546900

D D Belt
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PREVIOUS AND PRESENT PROGRAMS

	Sponsor	Program
1.	Commercial Sponsor	"Free Radicals in Paper by Electron Spin Resonance," 4-7-58 to 1-7-59.
2.	Quartermaster Corps	"Detection of Radiation Induced Free Radicals by Paramagnetic Resonance," Contract No. DA 19-129-QM-387, 5-18-55 to 10-18-57.
3.	Quartermaster Corps	"Detection of Radiation Induced Free Radicals by Paramagnetic Resonance," Contract No. 19-129-QM-1060, 11-14-57 to 7-13-59.
4.	Rome Air Development Center	"Investigation of Applications of Magnetic Resonance Absorption Spectroscopy to the Study of the Effects of Microwaves in Biological Materials," Contract No. AF 30-(602)-1843, 5-1-58 to 5-1-59.
5.	San Antonio R&D Procurement, Brooks AFB	"The Study of Radiation Induced Free Radicals in Chemical and Biological Systems," Contract No. AF 41(657)-246, 8-1-58 to 10-29-63.
6.	Southwest Research Institute	"Basic Research—Free Radicals in Biological Materials," 8-28-57 to 1-31-58.
7.	Thiokol Chemical Corporation	"Development of Rapid Methods and Techniques for Analysis of Solid Propellants Based on Nuclear and Electron Paramagnetic Resonance," 10-31-57 to 12-8-58.
8.	Thiokol Chemical Corporation	"Electron Spin and Nuclear Spin Resonance Studies of PBAA Binder Systems," 12-5-58 to 3-20-60.
9.	Commercial Sponsor	"Application of Magnetic Resonance Methods to the Study of Reaction Mechanisms Involving Free Radicals," 9-1-59 to 12-31-61.
10.	Quartermaster Corps	"Study of Radiation Induced Reactions in Food Constituents by Resonance Methods," Contract No. DA 19-129-QM-1740, 12-30-60 to 12-29-62.

1003546901

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

	Sponsor	Program
11.	San Antonio R&D Procurement Office, Brooks Air Force Base, Texas	"The Study of Radiation Induced Free Radicals in Chemical and Biological Systems," Contract No. AF 41(657)-407, 8-1-51 to 11-30-63.
12.	Commercial Sponsor	"Application of Magnetic Resonance Methods to the Study of Energy Conversion Processes of Photosynthesis," 9-1-59 to 12-31-62.
13.	Commerical Sponsor	"The Mechanisms and Inhibition of Enzyme Action by Electron Spin Resonance," 5-14-62 to 5-13-63.
14.	Southwest Research Institute	"An Electron Spin Resonance Study of Negative Ions of Porphyrins, 8-24-62 to 12-1-65.
15.	Department of Health, Education & Welfare, Public Health Service	"Study of Radiation Damage Mechanisms to Biological Materials," 10-1-65 to 9-30-68.
16.	Brooks Air Force Base, Texas	"Determination of Free Radical Content in Chemical Systems," Contract No. AF 41(609)-2771, 2-15-65 to 3-11-66.
17.	Brooks Air Force Base, Texas	"The Effects of Ionizing Radiation on Oxidation States of Biological Systems," Contract No. AF 41(609)-2816, 7-1-65 to 7-31-66.
18.	Food and Drug Administration	"Investigation of Chemical-Electrical Sensing Methods for the Determination of Organ Phosphate Insecticides," Contract No. PROC. 63-105 (Neg.) 6-3-63 to 6-2-64.
19.	U.S. Army Dugway Proving Ground	"Design and Development of Electron Capture and Flame Ionization Techniques for Tracing Biological Aerosols in the Field," Contract No. DA 42-007-AMC-82(R), 1-29-64 to 7-1-65.
20.	Wright-Patterson AFB, Ohio	"Analysis of Amino Acids by Gas Chromatography," Contract No. AF 33(615)-1823, 6-10-64 to 6-15-65.

1003546902

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

	Sponsor	Program
21.	Council for Tobacco Research	"The Correlation of Acetonitrile in Body Fluids to Tobacco Usage," 4-1-64 to 3-31-65.
22.	Southwest Research Institute	"Separation and Detection of Nitrogen Compounds of Biological Interest," 6-23-64 to 6-22-66.
23.	U. S. Department of Agriculture	"A Study of the Basic Methods of Action of Several Fungicides Using Radio Frequency Absorption Methods," Grant No. 12-14-100-802(34), 6-28-65 to 12-28-67.
24.	Public Health Service	"Radiation Damage Mechanisms in Biological Materials," Grant No. RH GM 00384-01, 1-1-65 to 9-30-68.
25.	National Institutes of Health	"Physical & Chemical Properties of Free Radicals and Alkylating Agents in Tobacco Smoke," PH43-65-100, 6-23-65 to 6-22-66.

1003546903

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

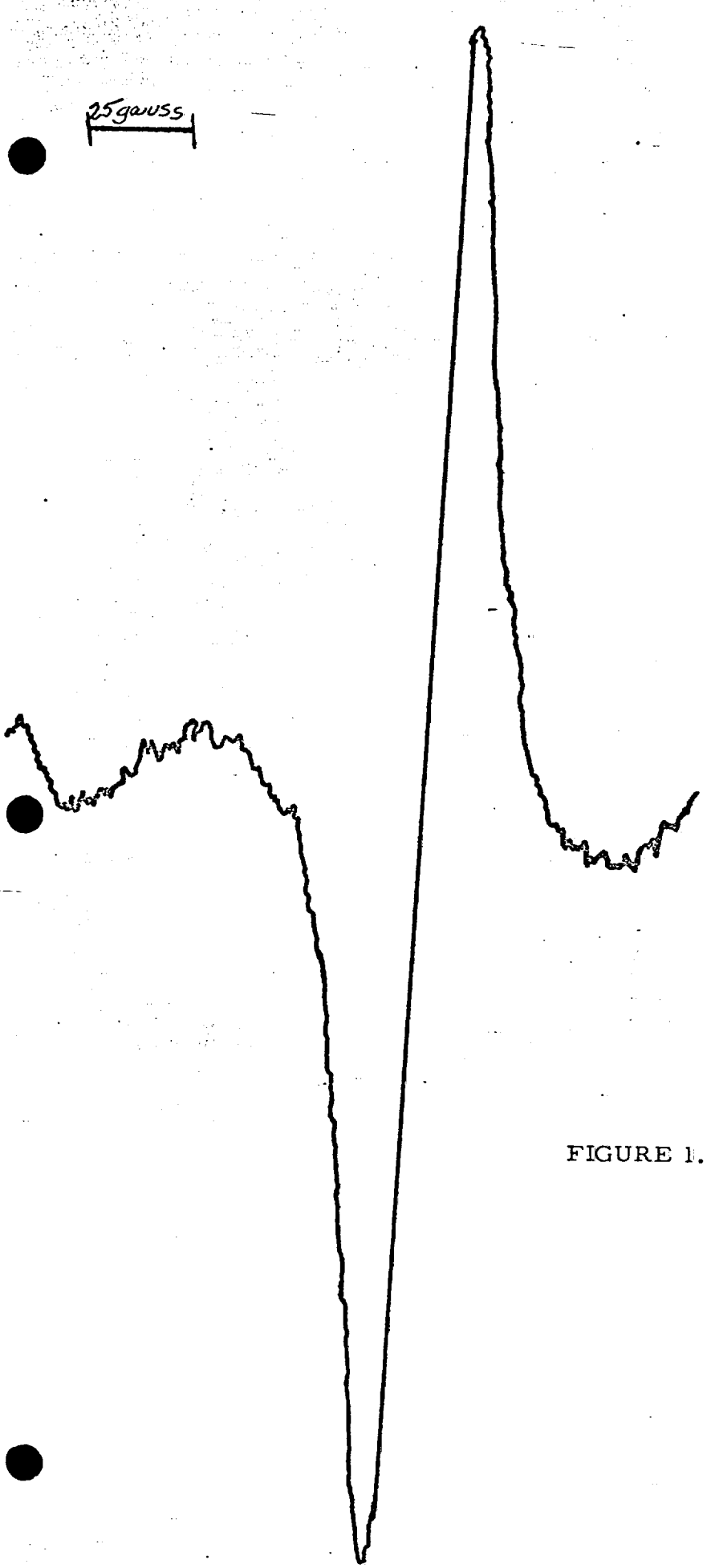


FIGURE 1. EPR OF TRAPPED CIGARETTE SMOKE.

10035469O4

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546905

FIGURE 2. EPR OF LUNG WHICH HAS SMOKED SIX CIGARETTES.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

SOUTHWEST RESEARCH INSTITUTE
8500 CULEBRA ROAD SAN ANTONIO, TEXAS 78206

March 3, 1967

Dr. Robert C. Hockett
Associate Scientific Director
The Council for Tobacco Research - U.S.A.
633 Third Avenue
New York, New York, 10017

Dear Dr. Hockett,

Here are, as promised, the electron spin resonance spectra of a hemoglobin solution which has been treated with the gaseous fraction of cigarette smoke. The spectra are quite similar to the ones contained in my proposal in which the total smoke condensate was allowed to react with the hemoglobin solution. These results strongly support the viewpoint I have already expressed in my proposal, that a detailed program involving the reactions of the constituents of the gaseous phase with selected enzyme systems be undertaken. I look forward to hearing from you.

Yours sincerely,

JOHN R. ROWLANDS
Staff Scientist, Department of
Physical and Biological Sciences

JRR/bjm

enclosures

1003546906

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

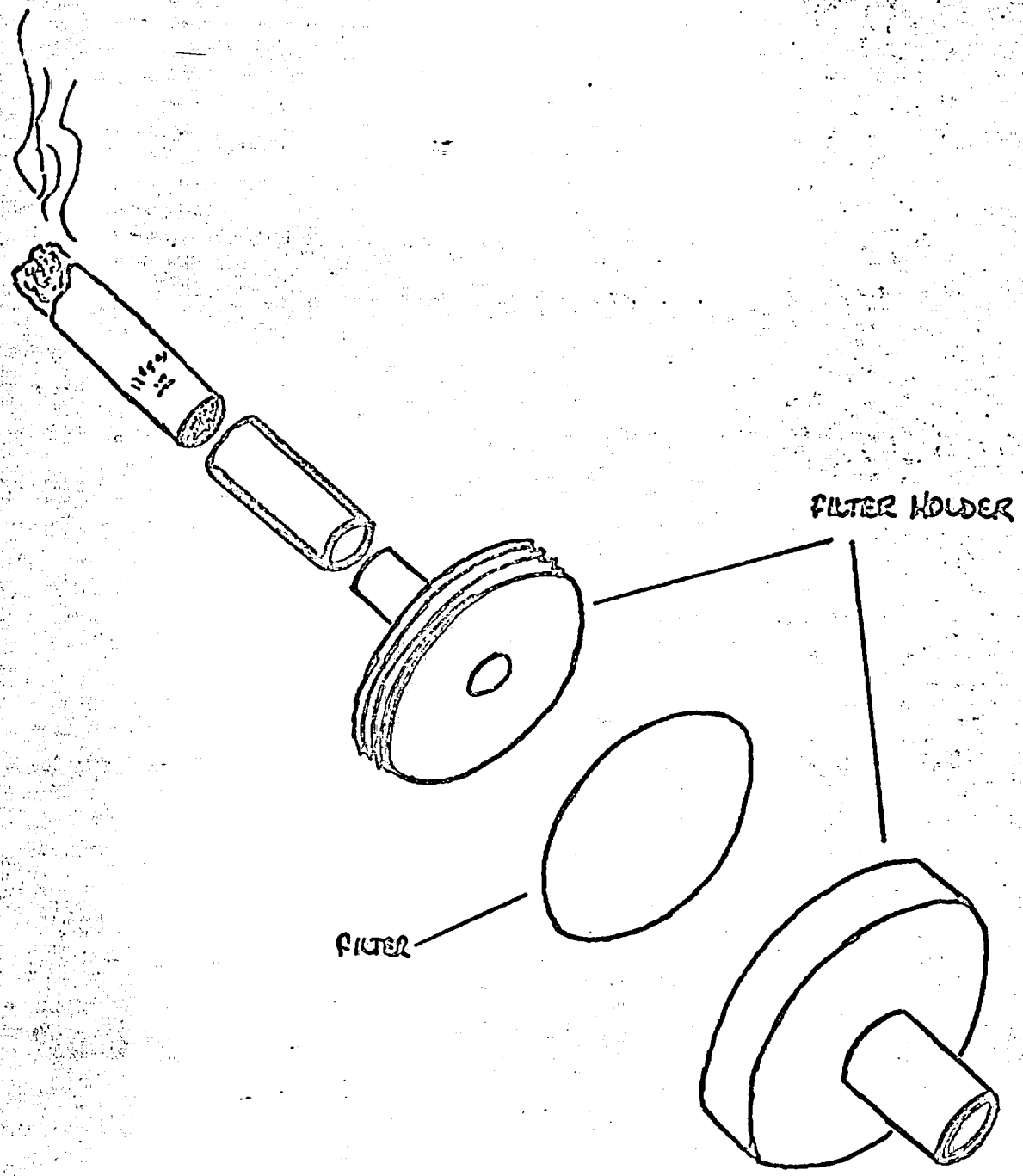
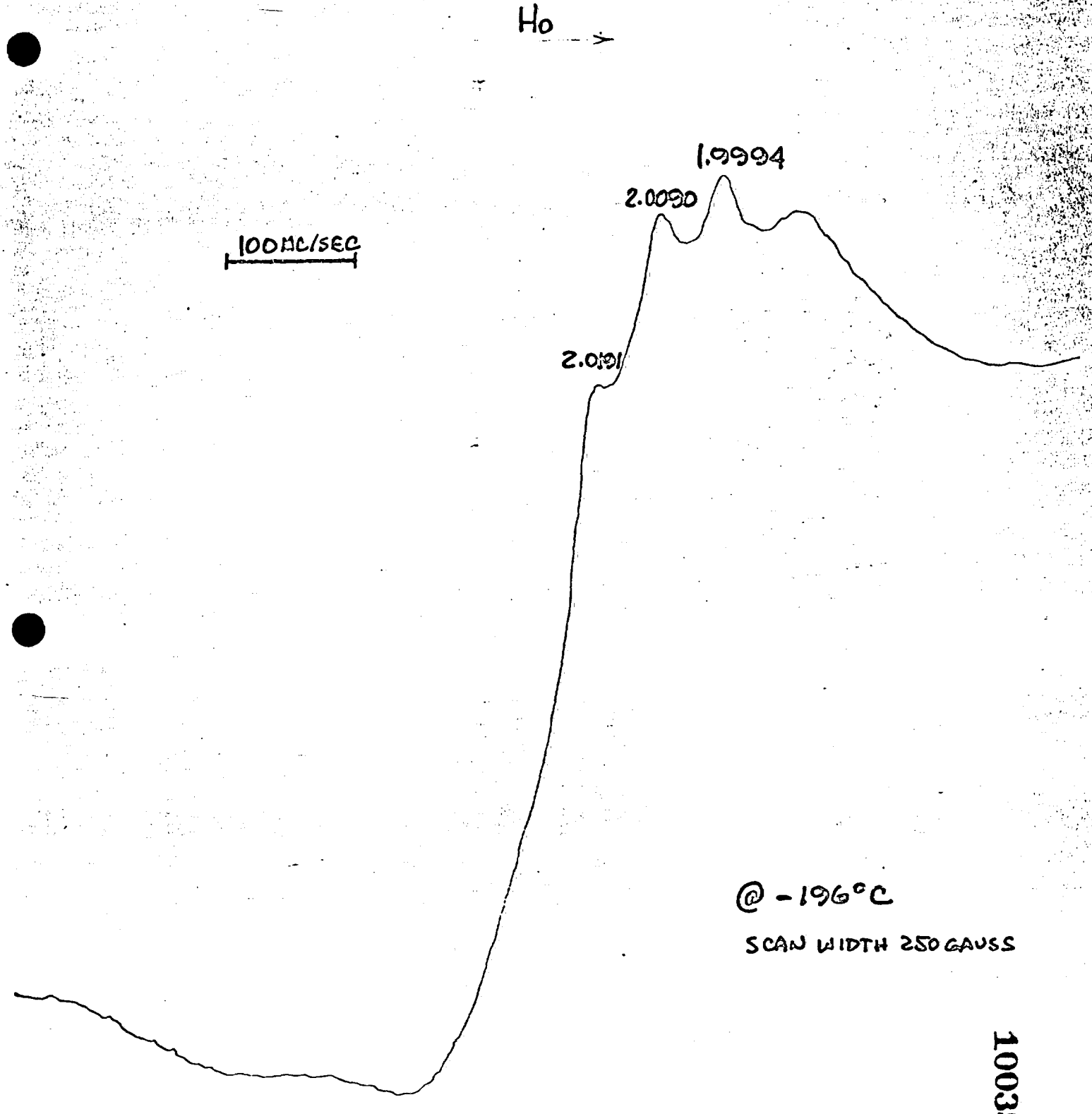


FIGURE 1 CIGARETTE SMOKE PARTICULATE MATTER FILTER

1003546907

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000



100354908

FIGURE 2 - ESR OF FILTERED CIGARETTE SMOKE REACTED WITH HEMIGLOBIN/DMSO. FIRST TEST

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

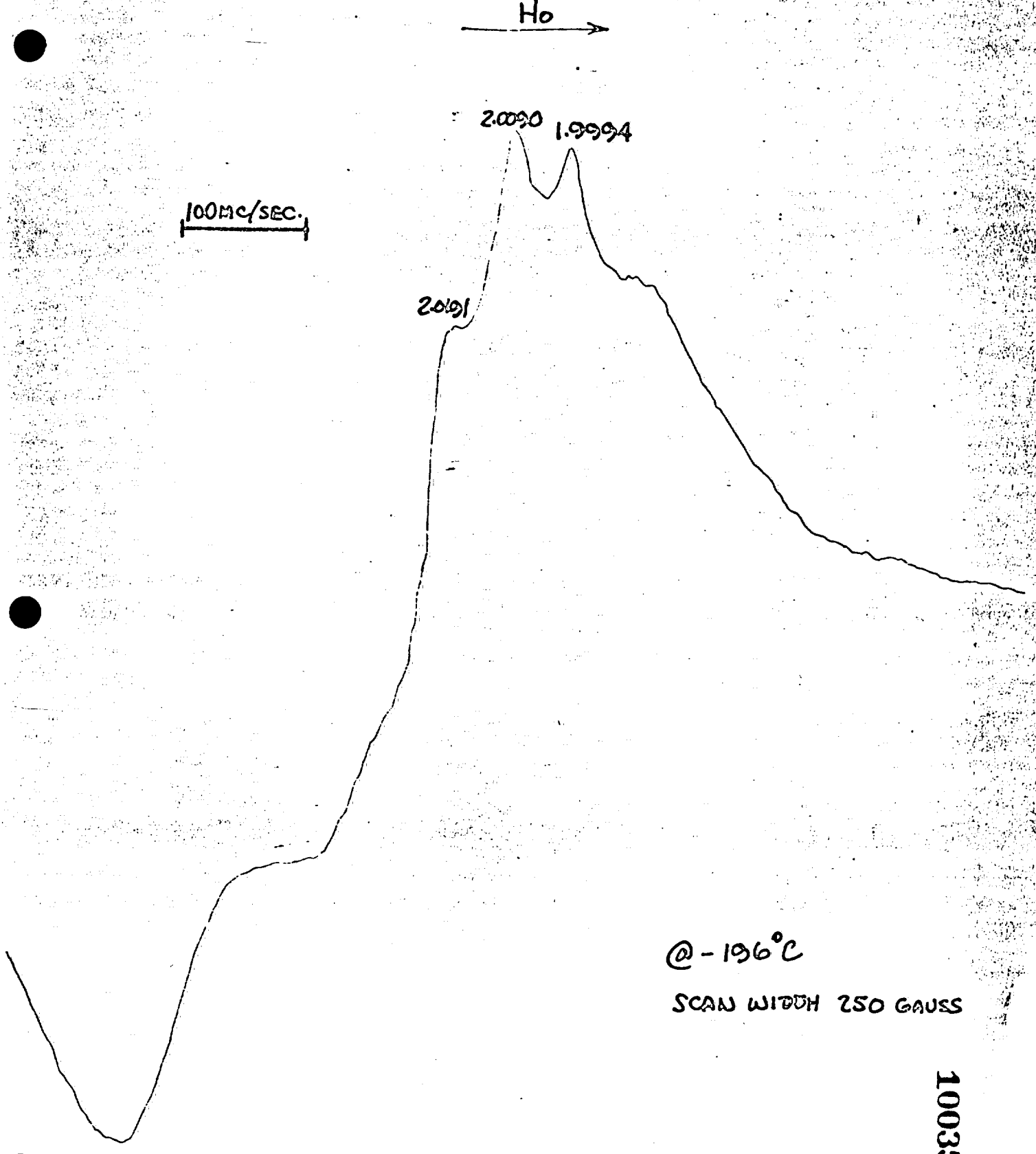
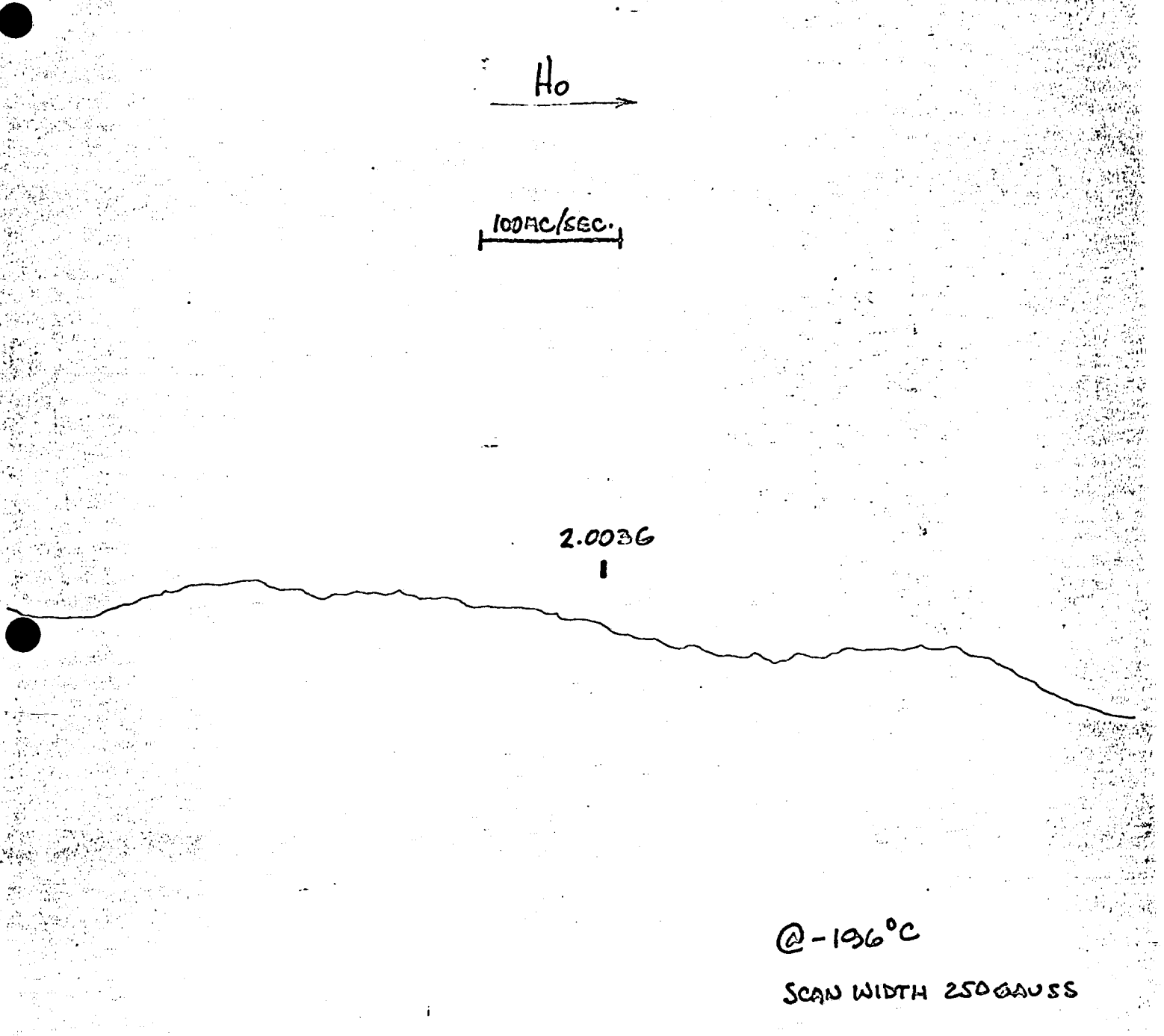


FIGURE 3 - ESR OF FILTERED CIGARETTE SMOKE REACTED WITH HEMIGLOBIN/DMSO. SECOND TEST.

1003546909

Source: https://www.industrydocuments.ucsf.edu/docs/pdm0000



100354910

FIGURE 4 - ESR OF HEMOGLOBIN/DMSO CONTROL

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

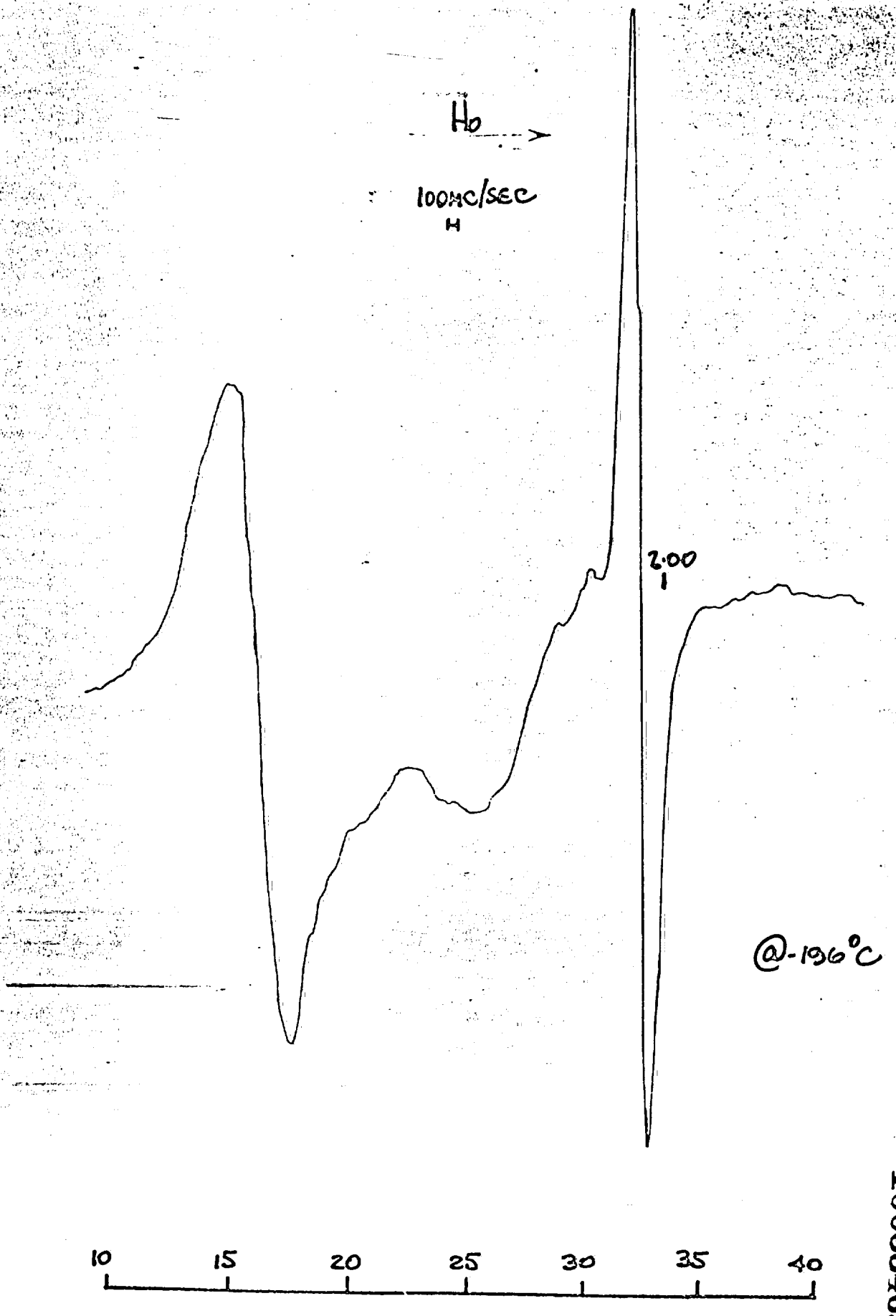


FIGURE 5 WIDE Ho; ESR SCAN OF FILTERED CIGARETTE SMOKE REACTED WITH HEMIGLOBIN/DMSO.

1003546911

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

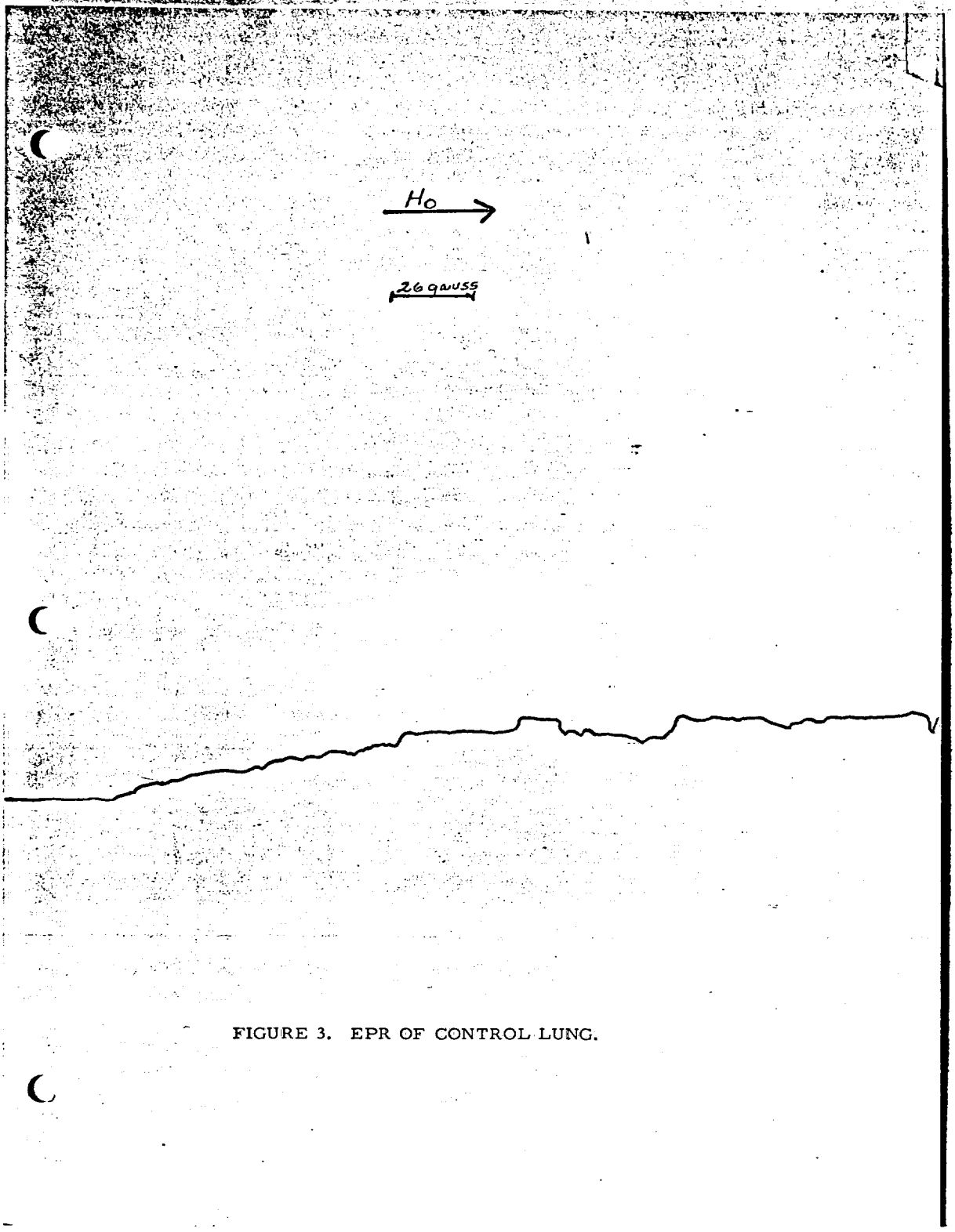


FIGURE 3. EPR OF CONTROL LUNG.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546912

August 3, 1966

Dr. J. Morrison Brady
Associated Scientific Director
The Council for Tobacco Research-U. S. A.
633 Third Avenue
New York, New York 10017

Dear Dr. Brady:

Several weeks ago I had a telephone conversation with one of your colleagues whose name I cannot recall. However the result of our conversation was that I was asked to submit some supplementary information concerning my grant request No. 5-4727. I hope the enclosed document covers the essential points we discussed and that it will meet with your review board's approval. If there is any point which I have overlooked or not explained sufficiently lucidly, please do not hesitate to write or telephone me for the extra information.

Yours sincerely,

John R. Rowlands
Senior Research Scientist
Department of Physical
and Biological Sciences

JRR/pm

Enclosures

1003546913

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Proposal
5-4727A
for

ESR STUDIES OF BIOCHEMICAL EFFECTS OF ATMOSPHERIC POLLUTANTS

to

THE COUNCIL FOR TOBACCO RESEARCH, U.S.A.
633 Third Avenue
New York, New York

Attention: Dr. J. Morrison Brady
Associated Scientific Director

August 3, 1966

1003546914

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

In our proposal #5-4727, I indicated that I had observed an electron paramagnetic resonance signal from an homogenate of a freshly excised rabbit lung into which had been smoked six popular brand cigarettes. A short pre-print of a publication, Appendix A, which outlines these results is included with this proposal supplement.

In a telephone conversation I was asked if I could give some additional information to strengthen my grant application. I believe the following discussion will supply the necessary information.

As indicated in Appendix A, I believe the observed electron paramagnetic resonance pattern is produced by the interaction of free radicals and/or reactive diamagnetic molecules with a metal complex present in the rabbit lungs. To be more specific I believe the bulk of the observed signal is due to the reaction of these free radicals and/or reactive molecules with the haemoglobin molecules present in the red blood cells. Such an interaction is represented in Figure 1. In the haemoglobin molecule the iron is present as a ferric ionic complex i.e. Fe^{3+}. The paramagnetic resonance spectra of Fe^{3+} ionic complexes have been investigated by many workers. In particular however, detailed electron spin resonance studies have been performed on four derivatives of the haemoglobin molecule, in order to discover the detailed form of bonding between the central iron atom and the particular group on the sixth coordination point, the point at which oxygen attachment occurs. **1003546915**

The tentative explanation of the reported ESR signal from smoked rabbit lung is the attachment of an active molecule (s) from tobacco smoke to the selfsame sixth coordination point. On the basis of this hypothesis several

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

extremely important questions arise:

1. Is this the transport mechanism to the liver which leads to the formation of acetonitrile by the liver?

2. Why does the lung membrane pass these molecules so readily?

3. What molecules or radicals are thus passed and picked up and are these identical to those in smoke?

4. Is there a connection between these modified haemoglobin molecules and the observed vessel constriction in smokers?

Since none of these questions lie within the scope of our present NCI program (primarily because of the unexpected nature of this discovery), TRC support is essential to allow the investigation of any or all of these. Before we attempt an exploration of the more complex biological questions raised above, there are several rather direct questions which must first be dealt with; for example:

1. What types of molecules and/or radicals will react to form haemoglobin complexes such as we have observed?

2. What is the stability of these complexes at physiological conditions?

3. Are such complexes formed by other atmospheric contaminents; i. e., smog, automobile exhaust fumes, etc. ?

1003546916

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THEORETICAL CONSIDERATIONS

The structure of the central portion of the haemoglobin molecule is represented in Fig. 1 where the haem-porphyrin plane is shown perpendicular to the axis joining the iron atom to the globin molecule and the ligand at the sixth coordination point. The main feature of the haemoglobin molecule is the relative ease with which the ligand of the sixth coordination point can be changed which makes it feasible to study a whole series of derivatives formed by substituting different ligands into this position.

Single crystal studies have been made on the acid methaemoglobin, acid metmyoglobin, metmyoglobin fluoride and metmyoglobin azide. The extreme g factors for each derivative are listed in Table I.

TABLE I

Derivative	Group attached	$g_{\parallel}$	$g_{\perp}$	μ in Bohr magnetons
Acid methaemoglobin	H_2O	2.0	6.0	5.84
Acid metmyoglobin	H_2O	2.0	6.0	5.85
Metmyoglobin fluoride	F	2.0	6.0	5.92
Metmyoglobin azide	N_3	2.8	1.70	2.84

The parallel suffix refers to directions parallel to the axis through the iron atom normal to the haem plane and the perpendicular suffix to directions at right angles to this axis (i.e., in the haem plane). It can be seen from Table I that the four derivatives so far studied are divided into two classes by both

1003546917

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

the resonance and susceptibility measurements. As a result of the susceptibility data these two groups were classified as "essentially ionic" and "essentially covalent" corresponding to the cases of a spin quantum number S = 5/2 or 1/2. Thus, if ionic binding is assumed, the central positively charged ferric ion will be left with five 3d electrons. These will line up with their spins parallel according to Hund's rule and so half fill each of the five vacant 3d orbitals. A total electronic spin moment of 5/2 is thus formed which will itself be quantized in any applied field. On the other hand, if covalent bonding takes place six pairs of electrons are required to form with the surrounding ligands. These electrons donated by the surrounding atoms will occupy certain orbitals of the ferric iron, and it can be shown that the most stable configuration for octahedral bonding is the one in which two 3d orbitals and the one 4s orbital and three 4p orbitals are employed. The five non bonding 3d electrons of the ferric iron are therfore, left with only three 3d orbitals to occupy and hence four are forced to pair leaving one unpaired and a resultant S = 1/2.

If this simple interpretation were correct the resonance spectrum obtained from the essentially ionic compounds would be expected to have the same general features as that of the other ionic ferric salts. Thus, the total spin and associated magnetic moment corresponding to the configuration with S = 5/2 would take up six different orientations varying from +5/2 to -5/2. Transitions can then be induced between each of these levels according to the selection rule $\Delta S = \pm 1$ and thus five electronic absorption lines would be expected centered on a g factor of 2.0. However, the work on single crystals of

1003546918

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

acid methaemoglobin, metmyoglobin and metmyoglobin fluoride showed that the g factor was in fact, anisotropic varying from a $g_{//}$ of 2.0 to a $g_{\perp}$ of 6.0, and that there was only one electronic transition observable. A theoretical explanation of the observed anisotropic values has been provided by assuming that very strong asymmetrical crystalline fields are active on the iron atoms so that the energy difference between the six different orientations of the total spin vector of 5/2 are much larger than the microwave quanta.

This highly anisotropic g factor of the "essentially ionic" haemoglobin derivatives makes the detection of the paramagnetic resonance signal of amorphous samples difficult, since the resonance line extends from approximately 1300 gauss to 3250 gauss. This of course, accounts for the each of any apparent signal from the sample of untreated rabbit lungs.

In contrast to the large variation from g = 2 to g = 6 observed for the ionic derivatives the azide derivative which is "essentially covalent" has a g factor spread across the free spin value, indicating an S of 1/2 with considerable orbital interactions. The signal we observe from rabbit lungs which have been treated with tobacco smoke is also located at approximately the free spin value. Consequently, we have tentatively attributed at least part of our observed signal to "essentially covalent" derivatives of haemoglobin being formed by reaction of the normal haemoglobin with constituents of the tobacco smoke.

1003546919

So far the above discussion o the nature of the resonance is hypothesis based upon what is known about iron in other complexes and has

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

to be verified experimentally. However, assuming the hypothesis is correct, such a complex provides a vehicle for carrying the chemicals present in cigarette smoke to the liver for the production of for example, acetonitrile.

1003546920

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PROPOSED PROGRAM

1. To establish that the origin of our observed electron spin resonance signal is in fact as we have outlined.

2. To determine the types of molecules and free radicals that will react with haemoglobin to produce the type of complex we have observed from the reaction of tobacco smoke with haemoglobin. In this study we will restrict ourselves to compounds which are normally found in tobacco smoke, smog, and automobile exhaust. Since all three systems contain many constituents in common, at this stage we will restrict ourselves to the investigation of representative examples of these common species.

3. Having established the necessary requirements for the production of such a complex, to investigate the stability of the complex, i.e., is the complex once formed stable or is the decomposition of the complex to reform undamaged haemoglobin a probable reaction?

4. To determine what effect the formation of such a complex has on the general reactivity of the haemoglobin molecule. Since the bonding of the iron atom to both the porphyrin ring and the globin molecule changes upon formation of such a complex this is accompanied by a change in electron distribution throughout the whole molecule. Such a change in electron distribution will necessarily cause changes in the chemical reactivities of different areas of this complex molecule.

1003546921

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

SUBMISSION OF REPORTS

A bi-annual report will be submitted to the Tobacco Research Council throughout the course of this program, with a final report summarizing the entire program. It is anticipated that much of this work will be appropriate for publication in the open Scientific Literature, but prior to publication the Sponsor requested to review the manuscript and give his consent to the publication.

1003546922

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REFERENCES

1. Ingram, Free Radicals as Studied by Electron Spin Resonance, Academic Press, (1958).

2. Swartz, et al, Biochem. and Biophysical Res. Commun., 21:61 (1965).

10035469 23

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Prepared by: John R. Rowlands

John R. Rowlands
Senior Research Scientist

Original signed by
Frank C. Whitmore

Approved: _____

Frank C. Whitmore
Deputy Director
Department of Physical and
Biological Sciences

Approved: A. C. Hulen

A. C. Hulen, Treasurer

10035469 24

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

APPENDIX A.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546925

THE EFFECTS OF TOBACCO SMOKE ON LUNG TISSUE AS MEASURED BY ELECTRON SPIN RESONANCE

by

John R. Rowlands, David G. Cadena, Jr. and Arthur L. Gross*
Southwest Research Institute
8500 Culebra Road
San Antonio, Texas

This work was supported by the National Cancer Institute, National Institutes of Health under Contract No. PH43-65-100.

*Present Address
Pico-Laboratories, Incorporated
8222 Broadway
San Antonio, Texas

1003546926

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

I. INTRODUCTION

The presence of free radicals in tobacco smoke has been previously reported.[1] During the course of a program concerned with free radicals and alkylating agents in tobacco smoke it was observed that the concentration of free radicals in tobacco smoke condensate varied considerably as a function of methods used for collecting and subsequent treatment with solvents. In order to determine the biological effects of free radicals it became apparent that a method was needed whereby smoke could be directly applied to lung tissue without first collecting and processing the condensate. The method reported herein enabled lung tissue to be directly exposed to cigarette smoke in a manner similar to normal smoking and resulted in the observation of distinct changes in the lung tissue as measured by electron spin resonance.

1003546927

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

II. EXPERIMENTAL

Large adult rabbits of unkown strain and obtained locally were used. Each animal was sacrificed by cervical dislocation and the lungs and the trachea were excised intact as rapidly as possible. This was accomplished within ten minutes after the animals were sacrificed. The trachea was then attached to the apparatus shown in Figure 1 using thin twine to hold it in place. The smoking apparatus (Figure 1) consists of a bell jar, valving, and a Phipps and Bird smoking machine that was modified to partially exhaust the bell jar, hold it in the exhausted state, and then admit atmospheric air into the jar. The timing of the cycle is controlled by the cam and motor arrangement contained in the smoking machine. The smoking parameters used were:

puff frequency	30 seconds
puff duration	2 seconds
inhalation time	4 seconds
butt length	15 mm

The puff volume varied with the capacity of the lungs during each experiment. The volume was adjusted so that the lungs would fully inflate without rupturing. During the initial phases of this work several lungs did rupture thereby allowing smoke to enter the bell jar. This was later prevented by first using the minimal puff volume that would inflate the lungs and step-wise increasing the volume until the lungs would inflate with a turgid appearance. King size, non-filtered cigarettes, manufactured in the United States were

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

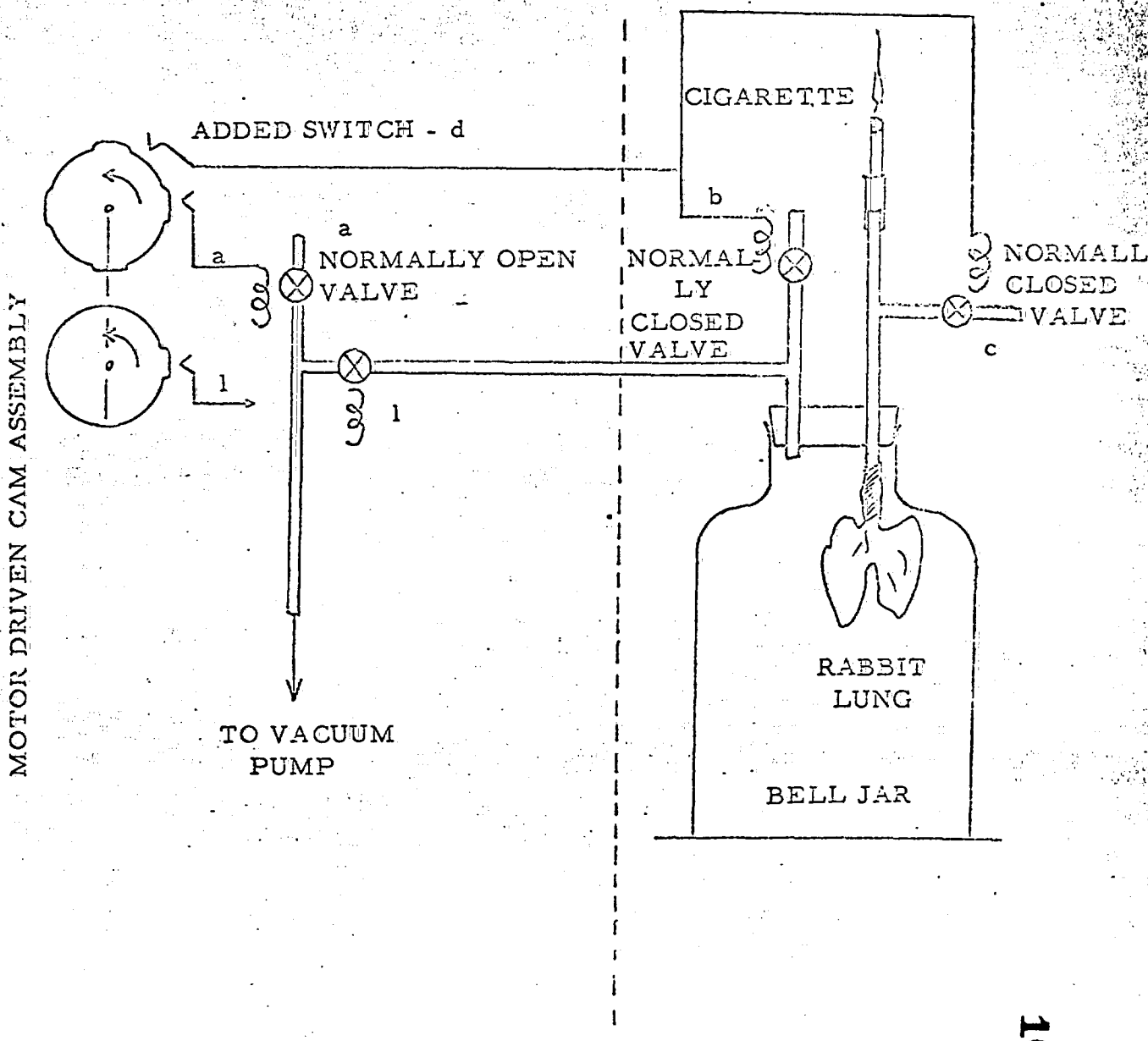


FIGURE 1. SMOKING APPARATUS

1003546929

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

used. The cigarettes were not preconditioned prior to use. They were inserted into a holder composed of rubber tubing and were ignited by means of a lit cigarette of the same brand. A total of six cigarettes were smoked into the lungs for each experiment.

As soon as the smoking was completed the lungs were removed from the apparatus and homogenized at room temperature for thirty seconds in an Omnimixer. A cylindrical sample of the homogenate was then prepared and placed into a Varian liquid nitrogen dewar. Electron spin resonance spectra of the sample were then recorded at 77° K using a Varian V 4500 X band spectrometer using 100 mc/sec modulation. A typical spectrum is shown in Figure 2 together with the spectrum obtained from an unsmoked lung using the same instrument parameters, and sample size. The observed spectrum was reasonably reproducible although small changes in the relative magnitudes of the three line pattern superimposed on the broad resonance line were noted from sample to sample indicating that the observed pattern is composite. A considerable amount of additional data is required before we can hope to arrive at any definite conclusions about the species giving rise to the signal. However, the similar pattern that Swartz[2] observed in irradiated blood suggests that the observed spectrum may be produced by the interaction of the free radicals in tobacco smoke with the red blood cells. This is being further investigated on further lung experiments and on a series of model systems including metal porphyrin complexes. In Figure 3 we have included the spectrum obtained from freeze dried smoke exposed lung tissue. Figure 3A represents the spectrum obtained at 77°K,

1003546930

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

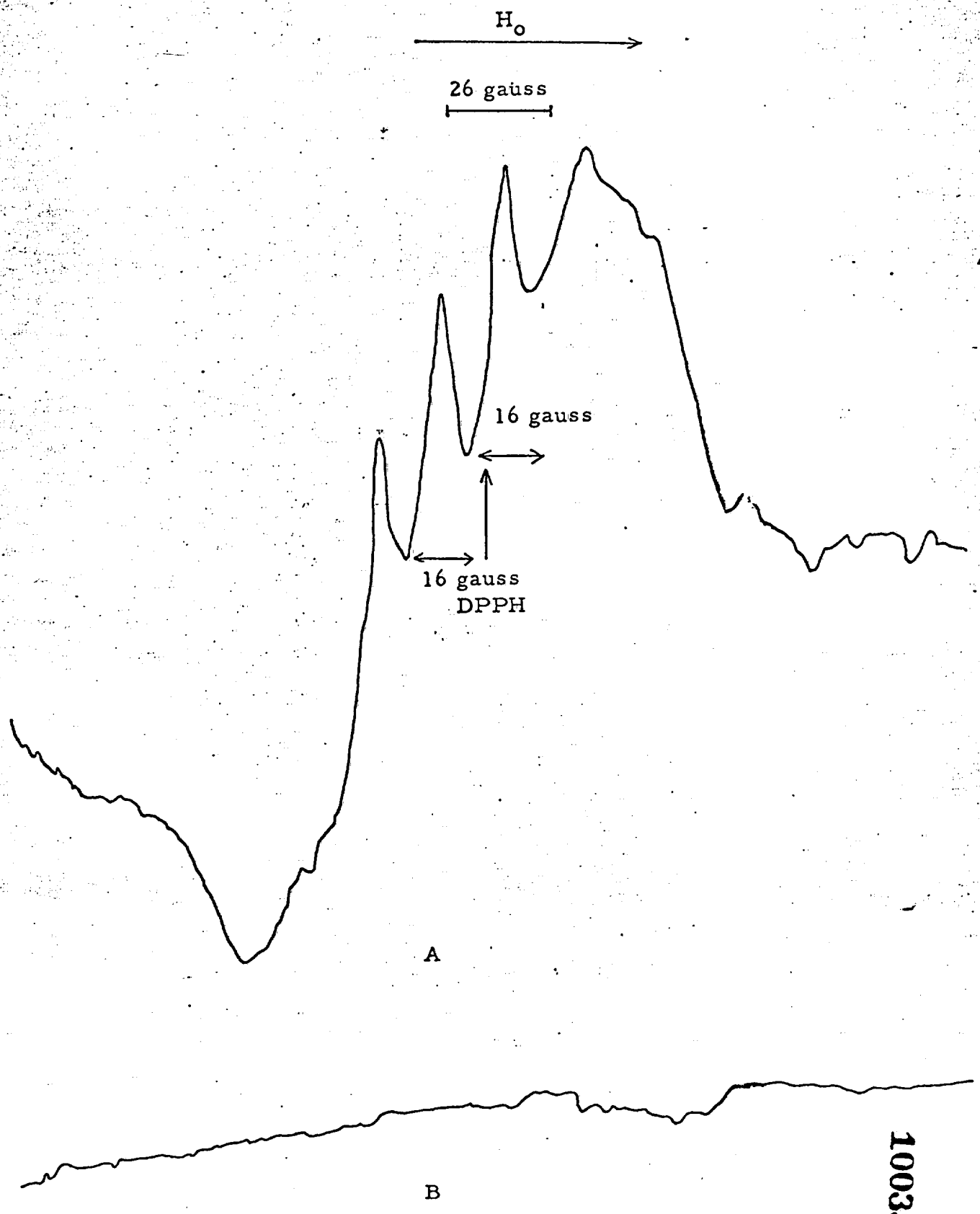


FIGURE 2. (A) ESR OF LUNG WHICH HAS SMOKED SIX CIGARETTES
(B) EPR OF CONTROL

1003546931

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

and Figure 3B the same sample run at room temperature. The room temperature signal consists of a single narrow line which is typical of the electron spin resonance signal obtained from tobacco smoke condensate. On refreezing to 77°K the electron spin resonance signal observed reverts to that shown in Figure 3A. The reappearance of the broad signal upon measurement at low temperature is consistent with it being due to a paramagnetic metal complex resonance, which becomes saturated and hence not detected at room temperature.

Conclusions

It is apparent from this work that the smoking system that is reported has proven to be a reliable method for exposing intact lung tissue to fresh cigarette smoke comparable to the manner of human exposure. The results are not biased by changes in the smoke that occur as a result of trapping and processing. We cannot at this time be certain of the origin of our observed electron spin resonance signal. However, due to the similarity of it to that observed by Swartz upon irradiation of red blood cells we anticipate that both his and our observations may be explained by free radical attack of the haemoglobin molecule at the sixth coordination position leading to a covalent hexacoordinated complex.

1003546932

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

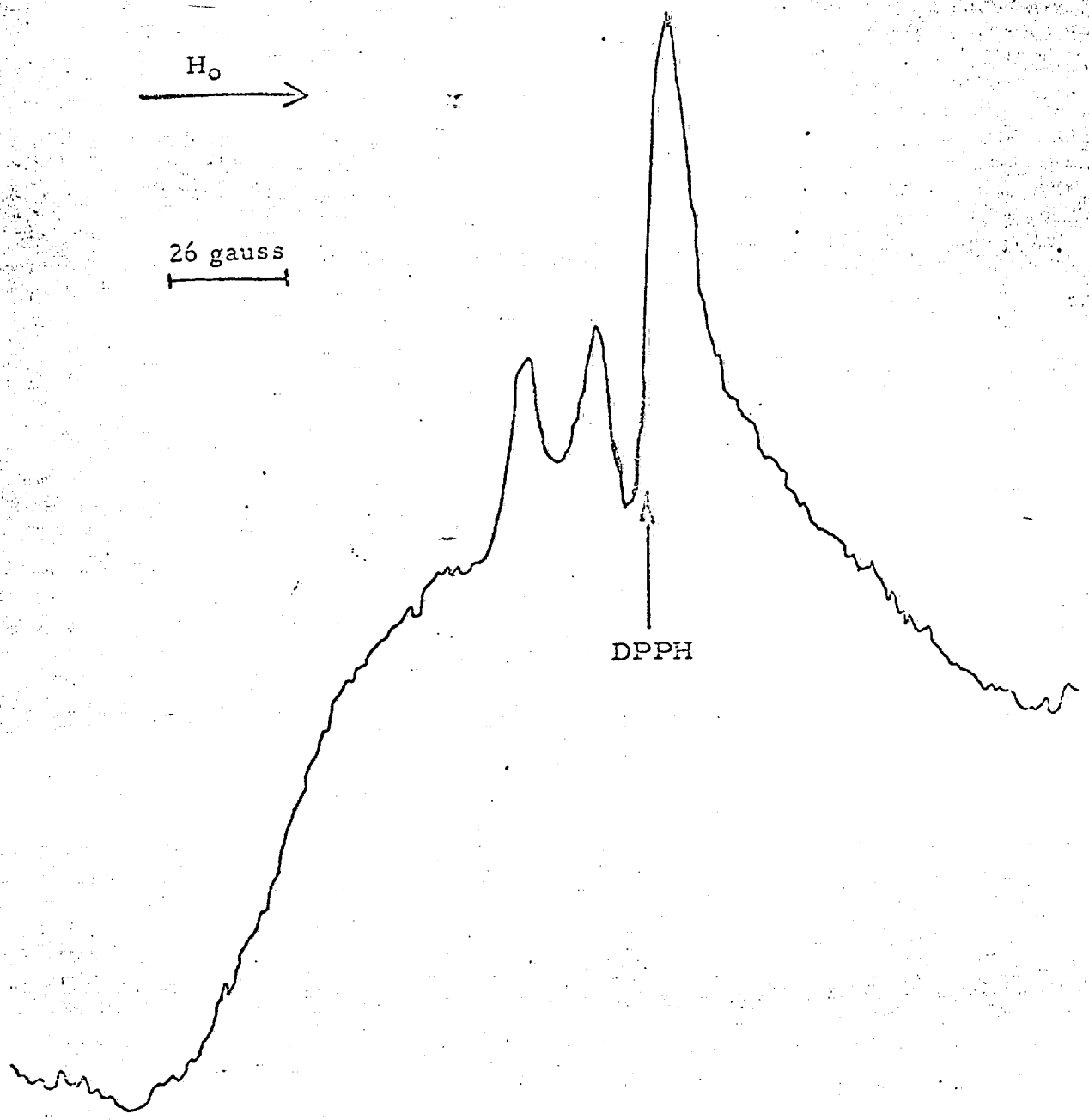


FIGURE 3A. EPR OF VACUUM DRIED SMOKED LUNG AT 77°K

10035469 33

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

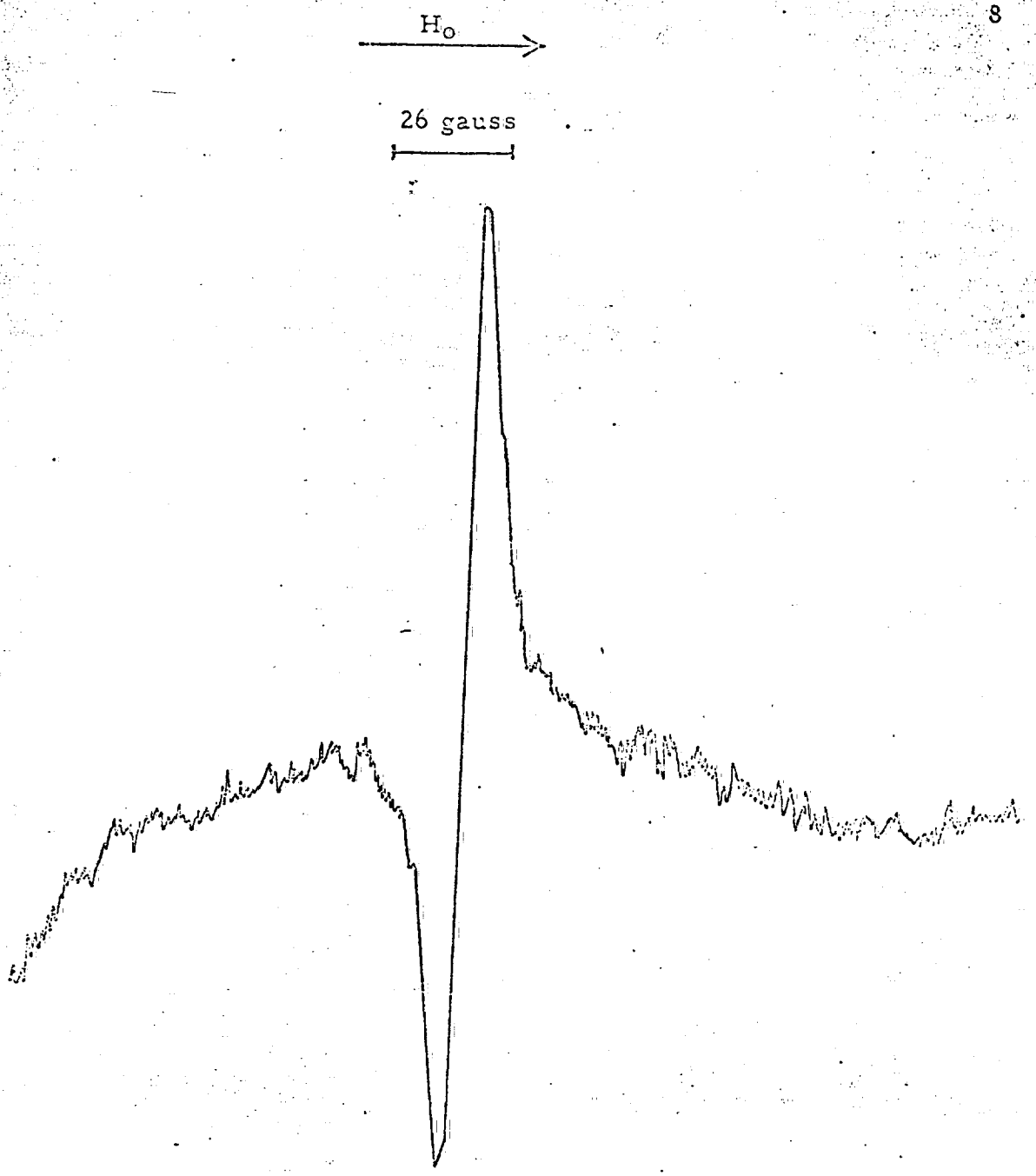


FIGURE 3B. EPR OF VACUUM DRIED LUNG AT ROOM TEMPERATURE

10035469 34

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

APPENDIX A.

10035469935

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PROPOSED PROGRAM

1. To establish that the origin of our observed electron spin resonance signal is in fact as we have outlined.

2. To determine the types of molecules and free radicals that will react with haemoglobin to produce the type of complex we have observed from the reaction of tobacco smoke with haemoglobin. In this study we will restrict ourselves to compounds which are normally found in tobacco smoke, smog, and automobile exhaust. Since all three systems contain many constituents in common, at this stage we will restrict ourselves to the investigation of representative examples of these common species.

3. Having established the necessary requirements for the production of such a complex, to investigate the stability of the complex, i. e., is the complex once formed stable or is the decomposition of the complex to reform undamaged haemoglobin a probable reaction?

4. To determine what effect the formation of such a complex has on the general reactivity of the haemoglobin molecule. Since the bonding of the iron atom to both the porphyrin ring and the globin molecule changes upon formation of such a complex this is accompanied by a change in electron distribution throughout the whole molecule. Such a change in electron distribution will necessarily cause changes in the chemical reactivities of different areas of this complex molecule.

1003546936

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

In our proposal #5-4727, I indicated that I had observed an electron paramagnetic resonance signal from an homogenate of a freshly excised rabbit lung into which had been smoked six popular brand cigarettes. A short preprint of a publication, Appendix A, which outlines these results is included with this proposal supplement.

In a telephone conversation I was asked if I could give some additional information to strengthen my grant application. I believe the following discussion will supply the necessary information.

As indicated in Appendix A, I believe the observed electron paramagnetic resonance pattern is produced by the interaction of free radicals and/or reactive diamagnetic molecules with a metal complex present in the rabbit lungs. To be more specific I believe the bulk of the observed signal is due to the reaction of these free radicals and/or reactive molecules with the haemoglobin molecules present in the red blood cells. Such an interaction is represented in Figure 1. In the haemoglobin molecule the iron is present as a ferric ionic complex i.e. Fe^{3+}. The paramagnetic resonance spectra of Fe^{3+} ionic complexes have been investigated by many workers. In particular however, detailed electron spin resonance studies have been performed on four derivatives of the haemoglobin molecule, in order to discover the detailed form of bonding between the central iron atom and the particular group on the sixth coordination point, the point at which oxygen attachment occurs.

The tentative explanation of the reported ESR signal from smoked rabbit lung is the attachment of an active molecule (s) from tobacco smoke to the selfsame sixth coordination point. On the basis of this hypothesis several

1003546937

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

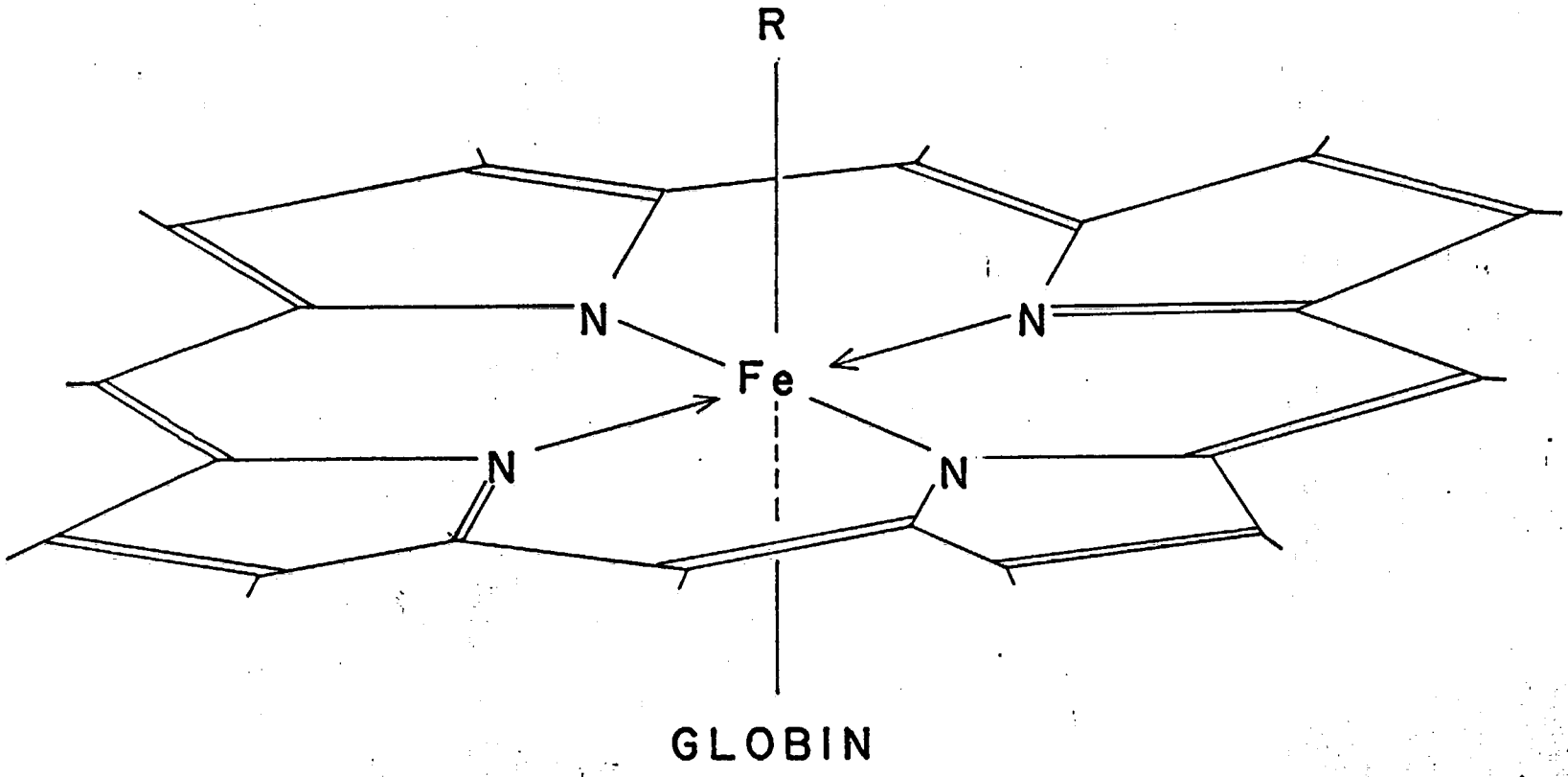


FIGURE 1.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546938

extremely important questions arise:

1. Is this the transport mechanism to the liver which leads to the formation of acetonitrile by the liver? deleted at the request of J.P. Rowlands

2. Why does the lung membrane pass these molecules so readily?

3. What molecules or radicals are thus passed and picked up and are these identical to those in smoke?

4. Is there a connection between these modified haemoglobin molecules and the observed vessel constriction in smokers?

Since none of these questions lie within the scope of our present NCI program (primarily because of the unexpected nature of this discovery), TRC support is essential to allow the investigation of any or all of these. Before we attempt an exploration of the more complex biological questions raised above, there are several rather direct questions which must first be dealt with; for example:

1. What types of molecules and/or radicals will react to form haemoglobin complexes such as we have observed?

2. What is the stability of these complexes at physiological conditions?

3. Are such complexes formed by other atmospheric contaminents; i.e., smog, automobile exhaust fumes, etc.?

10035469 39

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THEORETICAL CONSIDERATIONS

The structure of the central portion of the haemoglobin molecule is represented in Fig. 1 where the haem-porphyrin plane is shown perpendicular to the axis joining the iron atom to the globin molecule and the ligand at the sixth coordination point. The main feature of the haemoglobin molecule is the relative ease with which the ligand of the sixth coordination point can be changed which makes it feasible to study a whole series of derivatives formed by substituting different ligands into this position.

Single crystal studies have been made on the acid methaemoglobin, acid metmyoglobin, metmyoglobin fluoride and metmyoglobin azide. The extreme g factors for each derivative are listed in Table I.

TABLE I

Derivative	Group attached	$g_{\parallel}$	$g_{\perp}$	μ in Bohr magnetons
Acid methaemoglobin	H_2O	2.0	6.0	5.84
Acid metmyoglobin	H_2O	2.0	6.0	5.85
Metmyoglobin fluoride	F	2.0	6.0	5.92
Metmyoglobin azide	N_3	2.8	1.70	2.84

100354694O

The parallel suffix refers to directions parallel to the axis through the iron atom normal to the haem plane and the perpendicular suffix to directions at right angles to this axis (i.e., in the haem plane). It can be seen from Table I that the four derivatives so far studied are divided into two classes by both

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

the resonance and susceptibility measurements. As a result of the susceptibility data these two groups were classified as "essentially ionic" and "essentially covalent" corresponding to the cases of a spin quantum number S = 5/2 or 1/2. Thus, if ionic binding is assumed, the central positively charged ferric ion will be left with five 3d electrons. These will line up with their spins parallel according to Hund's rule and so half fill each of the five vacant 3d orbitals. A total electronic spin moment of 5/2 is thus formed which will itself be quantized in any applied field. On the other hand, if covalent bonding takes place six pairs of electrons are required to form with the surrounding ligands. These electrons donated by the surrounding atoms will occupy certain orbitals of the ferric iron, and it can be shown that the most stable configuration for octahedral bonding is the one in which two 3d orbitals and the one 4s orbital and three 4p orbitals are employed. The five non bonding 3d electrons of the ferric iron are therfore, left with only three 3d orbitals to occupy and hence four are forced to pair leaving one unpaired and a resultant S = 1/2.

If this simple interpretation were correct the resonance spectrum obtained from the essentially ionic compounds would be expected to have the same general features as that of the other ionic ferric salts. Thus, the total spin and associated magnetic moment corresponding to the configuration with S = 5/2 would take up six different orientations varying from +5/2 to -5/2. Transitions can then be induced between each of these levels according to the selection rule $\Delta S = \pm 1$ and thus five electronic absorption lines would be expected centered on a g factor of 2.0. However, the work on single crystals of

1003546941

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

acid methaemoglobin, metmyoglobin and metmyoglobin fluoride showed that the g factor was in fact, anisotropic varying from a $g_{//}$ of 2.0 to a $g_{\perp}$ of 6.0, and that there was only one electronic transition observable. A theoretical explanation of the observed anisotropic values has been provided by assuming that very strong asymmetrical crystalline fields are active on the iron atoms so that the energy difference between the six different orientations of the total spin vector of 5/2 are much larger than the microwave quanta.

This highly anisotropic g factor of the "essentially ionic" haemoglobin derivatives makes the detection of the paramagnetic resonance signal of amorphous samples difficult, since the resonance line extends from approximately 1300 gauss to 3250 gauss. This of course, accounts for the each of any apparent signal from the sample of untreated rabbit lungs.

In contrast to the large variation from g = 2 to g = 6 observed for the ionic derivatives the azide derivative which is "essentially covalent" has a g factor spread across the free spin value, indicating an S of 1/2 with considerable orbital interactions. The signal we observe from rabbit lungs which have been treated with tobacco smoke is also located at approximately the free spin value. Consequently, we have tentatively attributed at least part of our observed signal to "essentially covalent" derivatives of haemoglobin being formed by reaction of the normal haemoglobin with constituents of the tobacco smoke.

So far the above discussion of the nature of the resonance is hypothesis based upon what is known about iron in other complexes and has

1003546942

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

to be verified experimentally. However, assuming the hypothesis is correct, such a complex provides a vehicle for carrying the chemicals present in cigarette smoke to the liver for the production of ~~for example, acetonitrile~~ new compounds.

1003546943

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

SUBMISSION OF REPORTS

A bi-annual report will be submitted to the Tobacco Research Council throughout the course of this program, with a final report summarizing the entire program. It is anticipated that much of this work will be appropriate for publication in the open Scientific Literature, but prior to publication the Sponsor requested to review the manuscript and give his consent to the publication.

1003549944

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REFERENCES

1. Ingram, Free Radicals as Studied by Electron Spin Resonance, Academic Press, (1958).

2. Swartz, et al, Biochem. and Biophysical Res. Commun., 21:61 (1965).

10035469945

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Prepared by: John - R Rowlands

John R. Rowlands
Senior Research Scientist

Approved: H Whitmore

Frank C. Whitmore
Deputy Director
Department of Physical and
Biological Sciences

Approved: A. C. Hulen

A. C. Hulen, Treasurer

1003546946

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#622 GELLER

1003546947

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 1, 1967

Deferred to December meeting

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Little and Dr. Bing.

FROM: Robert C. Hockett

SUBJECT: New grant application from Irving Geller, Ph.D. - No. 622.

We enclose herewith a new grant application from Irving Geller, Ph.D. of Southwest Foundation for Research and Education, San Antonio, Texas.

The first portion has been already answered by Bonet et al (ref. to Morrison and Armitage, Effects of Nicotine on Behavior, N.Y. Acad. Sci. 142, 268 (1967) who observed that nicotine in moderate dosage facilitated the learning of the task and, under certain conditions, improved the performance of the rats.

The second section of this proposal would test the tranquilizing effect of nicotine, and whether nicotine dosage can be shown to allow an animal to overcome a negative (shock) stimulous in order to obtain food.

Comparison should be made to the application of Essman, #623.

Robert C. Hockett
John H. Kreisher

1003546948

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Source: https://www.industrydocuments.ucsf.edu/docs/pjcm0000

EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC NO. 622

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Little
Dr. Bing

Application For Research Grant

Date: 27 April 1967

1. Name of Investigator(s): (include Title and Degrees) Irving Geller, Ph.D.
Chairman, Department of Pharmacology and Toxicology

2. Institution & Address: Southwest Foundation for Research and Education
P.O. Box 2296 (10000 West Commerce Street)
San Antonio, Texas 78206

3. Short Title of Project: Effects of Nicotine on Learning in Laboratory Animals

4. Proposed Starting Date: 1 September 1967

5. Anticipated Duration of this Specific Study: Three Years

6. Brief Descripton of Objectives or Specific Aims: The basis for this research proposal stems from the notion that nicotine may exerta predominantly tranquilizing effect in man and animals (1). To test such a hypothesis, one must of necessity employ an experimental paradigm of problem solving, learning or some situation where the "tranquilized" or less anxious organism can perform at maximum capacity and ability. The experiments to be described in this proposal will illustrate the learning of a visual discrimination in hungry laboratory animals and some preliminary data which suggest that nicotine-injected animals may learn the task better than control animals. Such results might reflect a lowering of anxiety associated with the learning task and hence more rapid acquisition of the discrimination.

An extensive review of the literature reveals a paucity of research dealing with the effects of nicotine on learning or stress situations in animals (2,3,4,5,6). A comprehensive review of the existing studies has been published (7). A series of reports presented at a closed meeting in Colorado Springs (8) included a few investigations which dealt with the effects of nicotine on performance in laboratory animals. In such studies, subtle actions of nicotine tend to be overlooked and what usually results is a demonstration of the stimulant properties of nicotine. Investigators involved in such studies usually compare nicotine with known stimulants for effects on the performance of standard behavioral repertoires by animals.

A type of discrimination behavior is exemplified in a previous publication by this investigator and colleagues (9) who reported a delayed effect on behavior produced by small injections of nicotine bitartrate. In this experiment, hungry rats were trained to time precisely by rewarding with food those lever responses spaced 20 to 22 seconds apart. Injections of nicotine disrupted the timing behavior slightly, but pronounced delayed effects occurred three days after the drug injection and following a temporary return to base-line performance.

(Continuation on attached sheet)

7. Give a Brief Statement of your Working Hypothesis: "A consensus among modern authors appears to be that smoking and presumably nicotine exert a predominantly tranquilizing and relaxing effect(1)". The intent of this research is to test this hypothesis in a learning situation with laboratory animals. More rapid learning under nicotine could conceivably be attributed to a lowering of anxiety associated with acquisition of the assigned task.

1003546949

Objectives and Specific Aims (Continued)

It was conjectured that these delayed changes in behavior following nicotine injections could conceivably be related to epinephrine output. Silvette et al., in their extensive review (1), found it "difficult to ascribe the above reported effects to any pharmacological action of a single injection of nicotine three days previously. A more likely conclusion would seem to be that the animals were effected by some other part of the experimental procedure rather than the nicotine per se."

It is of interest to note that a later study (10) reported an increase in excretion of urinary epinephrine produced by chronic injections of nicotine. The excretion reached a peak after three days of treatment.

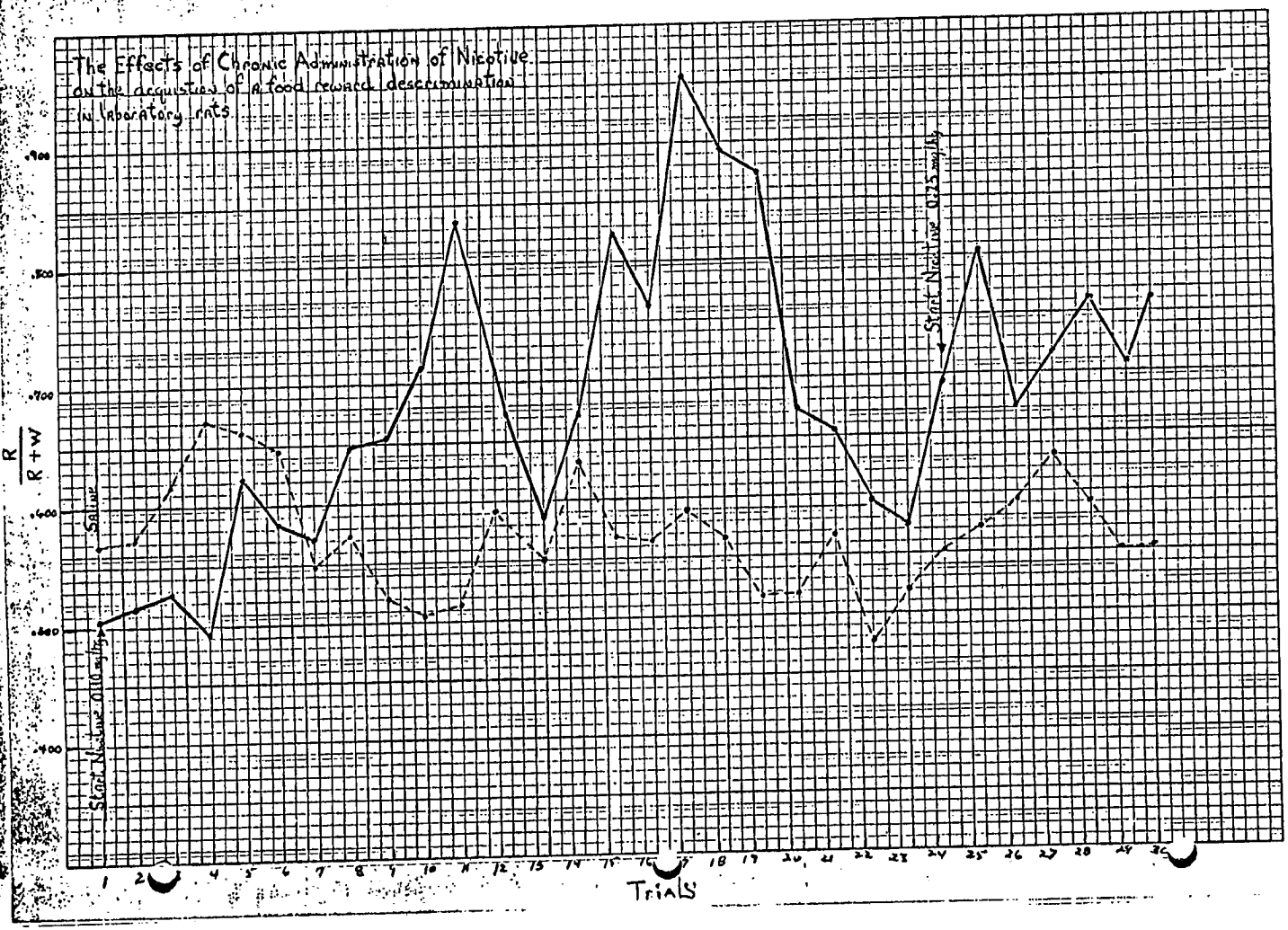
The intent of the proposed research is to measure rate of acquisition of discrimination behavior in laboratory animals who are administered nicotine chronically during acquisition training.

1003546950

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

5 x 5 TO THE ½ INCH
150GF GOLDSMITH BROS. N.Y. 9, CO 7-7900
MADE IN U.S.A.



1003596851

8. Details of Experimental Design and Procedures: (Attach Separate Pages) The subjects will be white rats, squirrel monkeys and cynomologous monkeys. They will be gradually reduced to 80% of their body weight and maintained at this weight by limited feedings throughout the course of the experiment. The apparatus will consist of two operant conditioning chambers for rats, conditioning chambers for squirrel monkeys and two Foringer monkey chairs.

Two experimental procedures will be employed. Procedure A will involve the effects of chronic administration of nicotine on the learning of a visual or auditory discrimination based on positive reinforcement (food reward). Procedure B will involve the effects of chronic administration of nicotine on the learning of an auditory discrimination based on punishment ("conflict").

Procedure A. Hungry animals will learn to press a lever in order to obtain a liquid or solid food reward. Once the animals learn that depressing the lever is associated with the delivery of food rewards, the experimental conditions will be as follows: random presentations of light stimuli in the experimental chambers will serve as a signal that a lever response will be incorrect and will be punished by a 30 second period of darkness for the animals. If however, a tone stimulus accompanies the presentation of the light stimulus, a lever response will be correct and the hungry animal will be reinforced with food. For some animals light alone will indicate an incorrect response, while light and tone will indicate a correct response. For another group of animals tone alone will indicate an incorrect response, while tone plus light will indicate a correct response. (See Attachment)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

The general facilities of the Southwest Foundation for Research and Education are available for this project. These include adequate office, laboratory and animal room space.

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append) Attached

12. List of publications: (Five most recent as pertinent) (append) Attached

10035469S2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Experimental Design and Procedures (Continued)

All experimental animals will receive chronic administrations of nicotine during acquisition training, while control animals will receive saline.

Treatment of Data: In Figure 1 are preliminary data for two of four experimental rats which illustrate nicotine effects on the acquisition of such a discrimination. On the ordinate is shown efficiency or how well the animal is performing. These points are determined by taking the ratio of right responses divided by right and wrong or $\frac{R}{R+W}$ = efficiency. If the animal is making an equal number of right and wrong responses, he is not discriminating and the point would fall at 0.50. If the animal's wrong responses exceed the right responses, the point would fall below 0.50. Points lying above .50 indicate that right responses exceed wrong responses and that the animal is acquiring the discrimination. The solid red line in the figure representing data for the nicotine rat shows that this animal acquired the discrimination faster than the saline animal. When the performance of the discrimination began to drop on the 20 to 23 day, the nicotine dose was increased from 0.1 to .25 mg/kg and the performance of the animal again became superior to that of the saline rat.

If, in the proposed experiment, the nicotine animals do acquire the discrimination more rapidly than the saline controls, it may be of interest to determine what effect the discontinuance of nicotine injections will have on the performance of the discrimination.

Possible Results:

1. Nicotine treated animals will acquire the discrimination more rapidly than saline controls.
2. Saline controls will acquire the behavior more rapidly than nicotine animals.
3. There will be no significant differences between nicotine and saline animals in the rate of acquisition of the discrimination.

Procedure B: This procedure, developed by this investigator and colleagues in 1957, is currently used extensively in the pharmaceutical industry for the pre-clinical evaluation of the minor tranquilizers (anti-anxiety agents). The behavior involves the learning of an auditory discrimination (or visual) based on punishment. The auditory discrimination is conditioned in hungry laboratory animals by simultaneously rewarding with food and punishing with mild electric shock all lever responses made in the presence of a discriminative stimulus (tone or light). Appropriate setting of the shock intensity results in suppression of ongoing lever pressing in the presence of the discriminative stimulus. The intent of this experiment is to investigate the rate of acquisition of such a discrimination in animals administered nicotine chronically.

The hungry laboratory animals first learn to press a lever in order to obtain food rewards which are obtainable on the average of once every two minutes (2 minute VI). When lever pressing rates have stabilized a tone or light stimulus of 3 minute duration is introduced at regular intervals during the lever-pressing session. This stimulus serves as a signal for the animal that all lever responses will be reinforced with food. The discriminative stimulus signals a change from a relatively undesirable schedule of reinforcement (2 minute VI) to a schedule with a higher "pay-off" of reinforcements (continuous reinforcement). When the behavior has stabilized, a

1003546953

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Experimental Design and Procedures (Continued)

punishment contingency is added so that during the discriminative stimulus, lever pressing is rewarded with food but also produces mild shock to the feet of the animal. The hungry animal must balance the positive aspects of obtaining food against the negative aspects of accepting electric shocks in order to obtain the food. After a period of time these experimental contingencies result in a suppression of lever-pressing behavior during stimulus periods. Anti-anxiety agents will reinstate lever pressing behavior that has been suppressed by punishment (11,12,13).

This investigation will test the effects of nicotine on the acquisition of such a discrimination in laboratory animals. Animals will be administered nicotine chronically during acquisition training and will later be compared with saline controls.

Possible Results:

1. Nicotine animals will acquire the punishment discrimination more rapidly than saline controls.
2. Saline animals will acquire the punishment discrimination more rapidly than nicotine animals.
3. There will be no significant difference between saline and nicotine animals in acquisition of the punishment discrimination.

The data from these experiments will show whether nicotine treated animals will acquire a discrimination more rapidly than saline controls. They will also show if the findings are similar for discriminations based on positive reward as well as punishment. They will yield information with regard to reproducibility of the phenomenon between species. Findings may or may not be qualitatively similar for rats, squirrel monkeys, and old world monkeys.

10035469S4

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REFERENCES

1. Silvette, H., Larson, P. S. and Haag, H. B.: Medical uses of tobacco past and present. Vir. Med. Monthly, 85, 472-484, 1958.

2. Smith, C. S., Rosenfeld, S. and Sacks, L. J.: Study of the effect of nicotinism in the albino rat. J. Pharmacol. 55, 274-287, 1935.

3. Kuschinsky, G. and Hotovy, R.: Uber die zentral erregende Wirkung des nicotins. Klin Wschr. 22, 649-650, 1943.

4. Heimstra, H. W., Grodsky, M. A. and Davis, R. T.: Sand digging behavior of rats. Proc. S. Dak. Acad. Sci. 34, 96-102, 1955.

5. Cervalo, B. A. and Cheskey, C.: Effects of nicotine sulfate injections on sand digging behavior of rats. Proc. S. Dak. Acad. Sci. 36, 222-225, 1957.

6. Bonta, I.L., Delver, A., Simons, L. and De Vos, C. J.: A newly developed motility apparatus and its applicability in two pharmacological designs. Arch. Int. Pharmacodyn. 129, 381-394, 1960.

7. Silvette, H., Hoff, E. C., Larson, P. S. and Haag, H. B.: The actions of nicotine on central nervous system functions, Pharm. Rev. 14, 137-173, 1962.

8. Research Conference and Workshop on Nicotine. Sponsored by AMA and held in Colorado Springs, November 1-3, 1966.

9. Geller, I., De Marco, A. O. and Seifter, J.: Delayed effects of nicotine on timing behavior in the rat. Science. 131: 1960

10. Westfall, T. C. and Watts, D. T.: The effect of nicotine on amines of brain and urine in the rat. J. of Neurochem. 11, 397-402, 1964.

11. Geller, I. and Seifter, J.: Effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia. 1, 1960.

12. Geller, I. and Seifter, J.: The effects of monourethanes, diurethanes and barbiturates on a punishment discrimination. J. Pharmacol. Exptl. Therapeutics. 136, No. 3, 1962.

13. Geller, I.: Use of approach avoidance behavior (conflict) for evaluating depressant drugs. Chapter 33, in Nodine (ed.), Psychosomatic Medicine, The First Hahnemann Symposium, Lea and Febiger: Philadelphia, 1962.

1003546955

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Irving Geller, Ph. D.

Date of Birth: [redacted] Place: Boston, Massachusetts.

Education:	Institution	Degree	Date	Major Field
	George Washington Univ.	B. A.	1949	Pre-med.
	George Washington Univ.	M. A.	1951	Psychology
	American University	Ph. D.	1957	Psychology

Present Position (s):

Chairman of the Department of Pharmacology, Division of Biological Growth and Development, Southwest Foundation for Research and Education. August 1966 - .

Previous Positions:

Aviation Psychologist - Naval Res. Lab., Washington, D. C.; Forbes Air Force Base, Topeka, Kansas, March 1951-November 1951.

Research Psychologist - Walter Reed Army Inst. of Research, Washington, D. C. 1952-1957.

Senior Research Scientist - Wyeth Labs. Inc., Radnor, Penna. 1957-1964.

Associate Research Professor of Pharmacology - New York Medical College, 1964 - 1966.

Professional Societies:

American Psychological Association
New York Academy of Science
Psychonomic Society
Sigma Xi
American Society for Pharmacology and Experimental Therapeutics.

10035469956

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS

Irving Geller, Ph. D.

1. Geller, Irving, M. Sidman and J. V. Brady, "The Effects of Electroconvulsive Shock on a Conditioned Emotional Response: A Control for Acquisition Recency," J. Comparative and Physiol. Psychol., Vol. 48, No. 2, 130-131 (1955).

2. Geller, Irving, and J. Seifter, "The Effects of Promazine and Phenergan on Multiple Schedule Reinforcement Performance in the Albino Rat," Fed. Proc., Vol. 18, (1959).

3. Geller, Irving, A. O. DeMarco and J. Seifter, "Delayed Effects of Nicotine on Timing Behavior in the Rat," Science, Vol. 131, No. 3402, 735-737 (1960).

4. Geller, Irving, and Joseph Seifter, "The Effects of Meprobamate, Barbiturates, d-Amphetamine and Promazine on Experimentally Induced Conflict in the Rat," Psychopharmacologia, 1, 482-492, (1960).

5. Geller, Irving, "The Acquisition and Extinction of Conditioned Suppression as a Function of the Base-Line Reinforcer," J. Expmntl. Anal. Behavior, Vol. 3, No. 3, 235-240 (1960).

6. Geller, Irving, "Behavioral Procedures Used in Evaluation of the Psychopharmacological Effects of Carphenazine," Diseases of the Nervous System, Supplement, Vol. XXII, No. 2 (1961).

7. Geller, Irving, and J. V. Brady, "Effect of Electroconvulsive Shock on an Extinguished "Fear" Response," Science, Vol. 133, No. 3458, 1080-1081 (1961).

8. Geller, Irving, "Use of Approach Avoidance Behavior (Conflict) for Evaluating Depressant Drugs," 1st Hahnemann Symp. on Psychosomatic Medicine, Chap. 33, 267-274 (1962).

9. Geller, Irving, J. T. Kulak, Jr. and J. Seifter, "The Effects of Chlordiazepoxide and Chlorpromazine on a Punishment Discrimination," Psychopharmacologia, 3, 374-385, (1962).

10. Geller, Irving, "Experimentally Induced Conflict for Evaluation of Psychopharmacologic Agents" (A Scientific Exhibit) (1962).

11. Geller, Irving, E. Bachman and J. Seifter, "Effects of Reserpine and Morphine on Behavior Suppressed by Punishment," Life Sciences, No. 4, 226-231 (1963).

12. Geller, Irving and J. Seifter, "The Effects of Mono-Urethans, Di-Urethans and Barbiturates on a Punishment Discrimination," J. Pharm. and Exptl. Therapeutics, Vol. 136, No. 3, 284-288 (1962) (one copy only).

13. Geller, Irving, "Conditioned "Anxiety" and Punishment Effects on Operant Behavior of Goldfish (Carassius auratus)." Science, 141, 3578, 351-353 (1963).

1003546957

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

14. DeMarco, A. O. and I. Geller, "Effects of Acceleration Forces on Timing Behavior in the White Rat," Aerospace Medicine, Vol. 35, No. 1, 30-32 (1964).

15. Geller, Irving, "Relative Potencies of Benzodiazepines as Measured by Their Effects on Conflict Behavior," Arch. int. Pharmacodyn, 149, No. 1-2, 243-247 (1964).

16. Geller, Irving, "Conditioned Suppression in Goldfish as a Function of Shock-Reinforcement Schedule," J. Experimental Analysis of Behavior, Vol. 7, No. 5, 345-349 (1964).

17. Geller, Irving, "Psychopharmacology of Tybamate," Journal of Psychopharmacology, Vol. 1, No. 2, 47-55 (1966).

10035469S8

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

3.

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Irving Geller, Ph. D.	25% (Salaries include fringe benefits)	
Technical		
Research Assistance	100%	R
Animal caretaker and Laboratory Helper	100%	R
	Sub-Total	R
B. Consumable Supplies (list by categories)		
Animals – Rats and Monkeys		
1. Animal food – wood shavings – syringes – vitamins – food reinforcers.		2500.00
2. Recorder paper, ink, pens, electrical supplies and replacement parts		500.00
	Sub-Total	3000.00
C. Other Expenses (itemize)		
Travel – visit to scientific meetings		500.00
Publication costs – illustrations, slides, etc.		500.00
	Sub-Total	1000.00
D. Permanent Equipment (itemize)		
Cages for housing squirrel monkeys and rats – includes devices for watering and feeding.		4500.00
Operant conditioning chambers for rats		1500.00
Operant conditioning chambers for squirrel monkeys (we have)		00.00
Behavioral Control equipment and recorders (see attachment)		8000.00
		14000.00
E. Overhead (15% of A+B+C)		2014.00
	Total	$29,442.00

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	$2000.00	$1200.00	$1500.00	$2438.00	$20,189.00
Year 3	R	$2300.00	$1000.00	$1800.00	$2535.00	$20,435.00

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Irving Geller PhD 5/2 OR 4-1410
Director of Project Telephone

Signature Edward F. Feith 5/2 OR 4-1410
Business Officer of the Institution Telephone
EDWARD F. FEITH
TREASURER

10035469S9

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Behavioral Control Equipment and Recorders

Qty	Description	Unit Price	Total Price
3	Cumulative Recorders	$ 450.00	$ 1350.00
3	4027 JM Reg. Power Supply	203.50	610.50
3	4005 J Timer	156.25	468.75
3	4005 JM Timer	193.75	581.25
6	4013 J Pulse Generator	51.25	307.50
3	4069 J Dual Pulse Generators	60.00	180.00
6	4001 J and Gate	43.75	262.50
6	4035 J Dual and Gate	43.75	262.50
1	4043 J Dual Exc. OR Gate	44.45	44.45
6	4011 J Diode Panels	30.00	180.00
6	4024 J Flip Flop R/S Binary	56.25	337.50
4	4072 J Basic Timer	101.25	405.00
3	4020 J Probability Generator	124.85	374.55
4	4023 J Delay Generator	58.75	235.00
6	4018 J Cradle Relay	31.00	186.00
6	4066 J Binary Relay (Alt.)	65.00	390.00
6	4068 J Session Timers	64.50	387.00
3	4028 J Pre Deter Counters	248.00	744.00
11	4010 J Reed Logic Relays	25.00	275.00
3	4025 J Noise Generator	139.50	418.50
	GRAND TOTAL		$ 8000.00

1003546960

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Experimental Studies in Psychopharmacology	NIH	$32,950 September, 1966 to August, 1967	Terminates August 31, 1967. Renewal approved but funds to be reduced more than 50%.
Effects of Nicotine on Timing Behavior in Rats	AMA	$25,000	May, 1966 - April, 1968

Pending

100354961

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546962

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 1, 1967

Defer until Dec. pending discussion re animal exposition

MEMORANDUM

TO: The committee comprising Dr. Little, Chm., Dr. Cattell and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New grant application from Walter B. Essman, Ph.D. - No. 623.

We enclose herewith a new grant application from Walter B. Essman, Ph.D. of Queens College of the City University of New York, Flushing, New York.

The proposed study is supported by some preliminary data reporting the effects of the rather large doses of nicotine employed on brain chemistry and retention of learning. It appears to be a sound scientific program and a competent investigator - and the main consideration may be whether or not we should support further work in this area in which we already have a number of projects.

The application from Dr. Irving Geller - No. 622, which will be on the agenda for the September meeting covers much of the same ground.

Robert C. Hockett

10035469G3

Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/

EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC NO. 623

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE
Dr. Little, Chm.
Dr. Cattell
Dr. Jacobson

Application For Research Grant

Date: June 30, 1967

1. Name of Investigator(s): (include Title and Degrees)

Walter B. Essman, Ph.D. - Professor

2. Institution & Address:

Queens College of the City University of New York
Kissena Blvd.
Flushing, N. Y. 11367

3. Short Title of Project:

Studies of Nicotine Action upon Memory Consolidation

4. Proposed Starting Date: October 1, 1967

5. Anticipated Duration of this Specific Study: Two years.

6. Brief Descripton of Objectives or Specific Aims:

The major objective of this proposed research is to assess the possible role of nicotine in determining the temporal character of memory consolidation in experimental animals. The proposed experiments represent an attempt to relate the effects of nicotine in the central nervous system to the behavioral processes which are dependent upon such central events. Specifically, the effect of nicotine on brain amine concentration and turnover will be related to the process of memory consolidation (the process by which the memory trace becomes fixated in the central nervous system; this may be empirically defined in terms of the time interval following learning within which memory for an acquired event may be disrupted by the introduction of external agents and/or events). Since the previous work in this laboratory has demonstrated that the temporal course for memory consolidation may be shortened or lengthened, depending upon changes brought about in brain amine levels, specifically serotonin, the studies proposed herein are based upon the assumption that nicotine-induced changes in brain amine levels will constitute the basis upon which memory consolidation may be facilitated. The parameters to be considered in the proposed studies are nicotine dosage and the intensity of the amnesic event which, for the present experiments, will be electroconvulsive shock. A one-trial conditioning technique will be utilized to establish a passive avoidance response, the retention of which will be tested, based upon the interaction of the effects of nicotine on the central nervous system and the effects of electroconvulsive shock both on the central nervous system as well as an amnesic event specific to the behavior concerned in the proposed studies.

7. Give a Brief Statement of your Working Hypothesis: Memory consolidation, as defined by the post-conditioning time interval, within which retention of a conditioned response may be disrupted by electroconvulsive shock, will be facilitated through the prior administration of nicotine. Such facilitation will be defined by a reduction in the time interval following learning, wherein electroconvulsive shock results in a subsequent retrograde amnesia for the conditioned behavior.

10035469 64

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See attached pages 5, 6, 7.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

A large air-conditioned laboratory with adjoining animal housing facilities is available and completely equipped with both behavioral testing apparatus and necessary equipment for biochemical procedures.

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

See attached page 10.

12. List of publications: (Five most recent as pertinent) (append)

See attached page 11.

10035469G5

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Technical		
Full-Time Research Assistant (To be selected)	100	R
Part-Time Research Assistant (To be selected)	50	R
Sub-Total		R
B. Consumable Supplies (list by categories)		
Animal purchases		1,200.
Animal food, bedding and cleaning supplies		600.
Chemicals and glassware		600.
Disposable cages		300.
Sub-Total		2,700.
C. Other Expenses (itemize)		
Photographic reproduction and duplication costs		200.
Reprint costs		300.
Sub-Total		500.
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)		1,770
Total		$13,570

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	$2,500	$500		$1,800	$13,800
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Councils "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Walter B. Casman
Director of Project

Signature [illegible]
Business Officer of the Institution

Telephone TU 6-6966

Telephone HI 5-7500 Ext.202

10035469 66

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Psychophysiological Properties of Thermal Change	National Institute of Mental Health	$20,050	6/1/66-12/31/67
	Role of Serotonin in Memory Consolidation	National Institute of Mental Health	4,200	9/1/66-8/31/67
Pending	Purine Metabolites in Memory Formation	National Institute of Mental Health	4,200	8/1/67-7/31/68

100354696?

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures:

All experiments will be based upon the use of a reliable technique which provides for the establishment of a conditioned passive avoidance response in mice, utilizing a single training trial (Essman & Alpern, 1965). The apparatus basically consisted of a small transparent lucite chamber fixed to a hole opening into a larger opaque chamber. The floor of the opaque chamber consisted of stainless steel grids which were wired in series through a cam-operated grid scrambler to a 95 volt power supply. Completion of the circuit by an animal stepping from the outer chamber into the inner one, through any 2 adjacent grids, provided for a 3.2 ma. shock delivered through the paws of the animal for 1 sec. The interval between placement of the animal in the outer chamber and its entry into the larger adjacent chamber was measured (response latency).

All animals utilized in the proposed experiments will be male, CF-1 strain mice (Mus Musculus), to be obtained from a commercial vendor (Carworth Farms, Inc.) at weaning (21 days), and following adaptation to conditions of laboratory housing (10 animals per cage), will be utilized as experimental subjects at approximately 30 days of age. They will eat each other up

The specific experiments will be concerned with:

1. Dose-response relationships for nicotine sulfate and rate of memory consolidation; the latter will be determined from the incidence of reduced retrograde amnesia induced by electroconvulsive shock (ECS).
2. The temporal gradient for ECS-induced retrograde amnesia in animals pretreated with nicotine sulfate.
3. The effect of nicotine sulfate on the incidence of conditioned response acquisition as a function of conditioning stimulus intensity.
4. The effect of nicotine sulfate on the incidence of conditioned response retention as a function of the intensity of the amnesic stimulus (ECS).

In the first series of experiments nicotine sulfate will be administered i.p. to mice in dose ranges of 0.25 mg/kg to 2.00 mg/kg (larger doses would not be indicated in view of neurotoxic effects). Control groups will be given an equivalent i.p. volume of 0.9% saline. Each group will be given a single training trial one hr. post-injection (stable CNS stimulant effect reached) in the apparatus described above, utilizing a 3.0 mA, 3 sec. training shock (yielding approximately 100% conditioned response acquisition), followed either immediately, 5, 10, 15, 20, 30, 45, or 60 min. by a 10 mA electroconvulsive shock (ECS), applied transcorneally for 200 msc. (sufficient to produce approximately 100% retrograde amnesia in control mice, when applied immediately post-training). A testing trial will be given to all groups of mice 24 hrs. following the training trial. Conditioned response retention will be evaluated on the basis of response latencies in the testing trial ($L \geq 30$ sec.) and retrograde amnesia will also be defined in terms of the absence of conditioned response as determined from latency measures ($L \leq 5$ sec.). Control groups, both drug and saline-treated, as described above, will be given no conditioning shock followed at the appropriate intervals by ECS, conditioning shock with subsequent sham-ECS (corneal electrodes applied, but no current passed), and no conditioning shock and sham-ECS. The outcome of the control group treatments is expected to be very similar to that obtained in pilot studies reported in the preliminary supporting data appended to this application. The data resulting from the proposed experiments will allow for conclusions regarding the dose-response relationship for nicotine sulfate and the rate of memory consolidation, determined from the incidence of reduced retrograde amnesia induced by ECS. Also, the temporal gradients for ECS-induced retrograde amnesia will be determined for nicotine sulfate. Since this

10035469G8

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

compound, within the proposed dose range, does not alter the susceptibility of mice to electroshock-induced convulsion, one may therefore assume that any change in the amnesic effect of electroshock is due to a central event rather than a threshold change. The usual temporal gradient for electroshock-induced amnesia has, in our experience, fallen within 1-hr. post-training with a reduced incidence of amnesia occurring as a function of the training electroshock time interval. From preliminary data it appears as though the gradient is appreciably shortened when animals have been pretreated with nicotine sulfate. The results of the proposed studies should conclusively establish the temporal boundaries of the amnesic effect of ECS.

The second series of proposed experiments will concern the effects of nicotine sulfate, to be utilized in the dose range specified above, upon the acquisition and retention of conditioned avoidance behavior as a function of (a) conditioning stimulus intensity (1.0, 2.0, or 3.0 mA) and (b) post-training ECS intensity (5.0, 10.0, or 20.0 mA). The incidence of conditioned response acquisition and the duration of stability of this response has been shown to increase as the conditioning shock intensity is increased, and the incidence of electroshock induced amnesia is increased as the ECS intensity is increased.

In the first experiment of this series animals will be conditioned, utilizing the technique described above and will be given either a foot shock of 1.0, 2.0, or 3.0 mA, applied through the grid floor upon successful completion of the response. Control animals in this situation will be given no conditioning shock. For each of these conditions a post-training electroconvulsive shock will be given at 5.0, 10.0, or 20.0 mA, either immediately following the training trial or at 10, 20, 30, or 60 minutes following the training trial. All animals will be tested for acquisition of the conditioned response as a function of the pre-training dosage of nicotine sulfate (0.25 mg/kg to 2.00 mg/kg), 24 hrs. following the training trial where the conditioning shock was varied. These data, for the effects of the drug upon retention as a function of the conditioning shock intensity, will be available from the non-convulsed control animals for which a saline-injected group will serve as a control for the drug dosage. Data relevant to the issue of post-training ECS intensity and the incidence of retrograde amnesia as a function thereof will be determined from nicotine sulfate and saline-treated groups given these injections one hour prior to the training trial. The testing trial will again be given 24 hours following training, and on the basis of the incidence of retrograde amnesia obtained statements with respect to the temporal gradient for retrograde amnesia as a function of an intensity of the amnesic event, electroconvulsive shock, may be made. On the basis of pilot studies it is anticipated that nicotine sulfate will reduce the temporal gradient for the amnesic effect of electroconvulsive shock, and that such a reduction will be a function of decreased ECS intensity as well.

Another series of experiments will be concerned with the biochemical effects of nicotine sulfate in the dose range which proves to be effective in facilitating memory consolidation, as determined from the experiments outlined above. Those specific biochemical changes with which the experiments will be concerned will deal primarily with the biogenic amines serotonin (5-hydroxytryptamine) and norepinephrine, as well as the substrates to which these amines are related. Since preliminary data has suggested that in the strain of mouse used there are no appreciable changes over time in norepinephrine, this observation will again be verified experimentally and the majority of the chemical determinations to be carried out will concern themselves with serotonin metabolism; in this connection, 5-hydroxytryptophane, the serotonin precursor and 5-hydroxyindoleacetic acid, the serotonin metabolite, will be assayed,

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

utilizing spectrofluorometric procedures which have been well standardized in this laboratory. Through these means serotonin concentration can be determined and reliable estimates of serotonin turnover may be obtained. These chemical determinations will be carried out under conditions of both experimental as well as control drug treatment and under conditions wherein electroconvulsive shock is administered or controlled for by sham treatment. It is anticipated that as a result of electroconvulsive shock whole brain serotonin concentration in control animals will be elevated. This hypothesis is based upon previous observations in this (Essman, W.B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113) and other laboratories (Kato, L., et al. Histamine, serotonin, epinephrine and norepinephrine in the rat brain, following convulsions. Int. J. Neuropsych., 1967, 3, 46-51). It is further hypothesized that the degree to which electroshock elevates brain serotonin will be limited under conditions where nicotine sulfate results in decreased brain serotonin levels as a function of dosage and post-injection time, and that under these conditions either serotonin concentration or serotonin turnover may be statistically correlated with the behavioral data indicating the incidence under these conditions of conditioned response retention; i.e., facilitated memory consolidation, inferred from nicotine sulfate-induced attenuation of electroshock-induced retrograde amnesia, will be positively correlated at those dosages and post-injection times where maximal changes in brain serotonin concentration and/or turnover are observed.

The proposed experiments may be summarized as an attempt to explore the relationship between the effects of nicotine sulfate on amine metabolism in the central nervous system and the process of memory consolidation based upon the hypothesis that nicotine-induced alterations in brain amine levels may account for a nicotine-induced facilitation of memory consolidation.

1003549970

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Preliminary Supporting Data

Several experiments completed in this laboratory have indicated the feasibility of the experiments proposed. The first of these studies was a series concerned with the acquisition by mice of a simple maze response requiring a choice discrimination in order to escape from a water maze. Groups of animals were given either 0.9% saline, 0.25 mg/kg, 0.50 mg/kg, or 1.00 mg/kg of nicotine sulfate, intraperitoneally. 15 min. following injection all animals were given 4 training trials spaced at 15-min. intervals in a water maze. Their escape times and incidence of errorless performance in the acquisition of the escape task were recorded. When the escape times for all groups of animals were linearly transformed across all trials in order to derive a decrement score indicative of the rate of maze acquisition, the only group reaching statistical significance was that comprised of those mice which were given 1.00 mg/kg of nicotine sulfate 15 min. prior to the first training trial ($t = 1.92$; $p < .05$). A comparison between groups for the incidence of errorless performance throughout the acquisition of the escape response indicated that there was a significant difference between groups in errorless performance and that this significance was largely accounted for by the high incidence of errorless performance among those animals receiving 1.00 mg/kg of nicotine sulfate ($\chi^2 = 4.95$, df = 1, $p < .02$). There was a 55% incidence of errorless performance among animals treated with 1.00 mg/kg of nicotine sulfate, as compared with an average of 28% errorless performance among the other groups in this study. These findings suggested that 1.00 mg/kg of nicotine sulfate exerted a significant effect in facilitating the acquisition of a simple escape response by mice.

Subsequent studies were concerned with the effect of a 1.00 mg/kg dose of nicotine sulfate in relation to the memory consolidation process. In these studies animals were treated with either nicotine sulfate or physiological saline 60 min. prior to being given a single training trial to establish a conditioned avoidance response. The training trial was followed immediately by a 10 mA electroconvulsive shock administered transcorneally for 200 msec.; 24 hrs. following the training trial all animals were tested for the incidence of retention and/or retrograde amnesia. Saline-treated animals showed between 90% and 100% incidence of retrograde amnesia, as defined by the absence of any conditioned avoidance behavior, whereas approximately 66% of the nicotine sulfate-treated animals showed retention of the conditioned avoidance response, with the remaining 34% showing the same retrograde amnesia for the conditioned avoidance behavior, as was shown by almost all of the saline-treated control animals. This finding suggested that the amnesic effect of electroconvulsive shock was either attenuated by nicotine sulfate treatment or that the time interval required for fixation of the memory trace was appreciably shortened through the effects of nicotine sulfate and was thereby less vulnerable to the amnesic effects characteristically exerted by post-learning electroconvulsive shock. The first alternative suggested by these data does not seem to appear dependent upon any alteration in the threshold to convulsion produced by electroshock since, for the dosage used, independent experiments established that for electroshock, varied from 5 through 20 mA, there was no alteration in the incidence of a full clonic-tonic convulsion following such electroshock administration at intervals from 15 min. to 60 min. after injection of 1.00 mg/kg of nicotine sulfate.

100346971

In another study where the interval between administration of 1.00 mg/kg of nicotine sulfate and the training trial, immediately followed by electroshock, was varied from 15 min. through 60 min., the data indicated that only when the compound was administered 60 min. prior to conditioning trial did a significant ($p < .01$) incidence of conditioned response retention occur. These data were consistent with those obtained in the previous study in that comparable results emerged, further

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

supporting the observation that nicotine sulfate exerts a peak effect upon memory consolidation when its central action occurs one hr. following administration of 1.00 mg/kg to mice. This observation is supported by some biochemical data in which, for saline-injected animals or mice treated with 1.00 mg/kg of nicotine sulfate at various intervals preceding the removal of brain tissue, whole brain serotonin and norepinephrine were extracted and assayed spectrofluorometrically. Contrary to a recent report (Westfall, T.C., et al. Effect of nicotine and related substances upon amine levels in the brain. Ann. N.Y. Acad. Sci., 1967, 142, 83-100) no changes in norepinephrine were observed as a function of post-injection time up to 60 min. post-injection, whereas whole brain serotonin did generally show a trend toward a decrement as a function of time. These data and the behavioral data previously referred to are summarized in Table 1.

Table 1

Mean Whole Brain Serotonin Concentration and Per Cent Incidence of Conditioned Response Retention after Electroshock for Mice given 0.9% Saline or 1.00 mg/kg of Nicotine Sulfate at Varying Intervals Prior to Conditioning.

Condition	Mean Whole Brain Serotonin	σ	Per Cent Incidence of CR Retention after ECS
0.9% Saline	0.677	0.089	10
Nicotine Sulfate (1.00 mg/kg)			
15 min.	0.676	0.058	10
30 min.	0.688	0.107	20
45 min.	0.655	0.083	25
60 min.	0.640	0.057	66

These data are consistent with a previous suggestion (Essman, W.B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113) that a reduction in brain serotonin level or pharmacologically-imposed limitations on the rate of serotonin turnover in the brain tend to limit the degree to which amnesic agents or events become effective in imposing a retrograde amnesia, and that these observations may be interpreted as a facilitation of the memory consolidation process whereby the time interval following learning, wherein the memory trace is normally vulnerable to the disruptive effects of known amnesic agents or events, is appreciably shortened by those conditions which tend to lead to a reduction in or limitation on the activity of brain serotonin. It is felt that nicotine sulfate is one such agent which, at the dosage utilized, provides for a wide margin of safety insofar as neurotoxic effects are concerned.

10035469?2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Biographical Sketch:

WALTER B. ESSMAN

Education:

Ph.D. - University of North Dakota, 1957
M. A. - University of North Dakota, 1955
B. A. - New York University, 1954

Experience:

Instructor, Dept. of Neuropsychiatry, U.S. Army Medical Service School, 1958-59.
Director, Psychophysiological Research, U.S. Army Surgical Research Unit, 1958-59.
Senior Post-Doctoral Fellow, Neurophysiology, Albert Einstein College of Medicine, 1959-61.
Research Associate, Dept. of Physiology, Albert Einstein College of Medicine, 1961-62.
Assistant Professor, Queens College, 1962-64.
Associate Professor, Queens College, 1965-66.
Professor, Queens College, 1967 - Present.
Research Fellow, Laboratory of Neurochemistry, Mt. Sinai Hospital, New York, 1964-66.
Research Associate, Laboratory of Neurochemistry, Mt. Sinai Hospital, New York, 1966 - Present.
Lecturer (Neurochemistry), Columbia University, 1964 - Present.

Areas of Major Research Interest:

1. Biochemistry of the central nervous system.
2. Neurochemical correlates of behavior.
3. Memory processes.

Memberships:

American Psychological Association
American Physiological Society
American Society of Zoologists
New York Academy of Sciences
American Association for the Advancement of Science

Honor Societies:

Psi Chi
Phi Delta Kappa
Alpha Pi Zeta
Sigma Xi

1003549973

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

12. Most Recent and Pertinent Publications:

Essman, W. B. and Alpern, H. Single trial learning: methodology and results with mice. Psychol. Rep., 1964, 14, 731-740.

Essman, W. B. Effect of tricyanoaminopropene on the amnesic effect of electroconvulsive shock. Psychopharmacologia, 1966, 9, 426-433.

Essman, W. B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113.

Essman, W. B. Changes in locomotor activity and brain chemistry following LSD-25 administration in mice. Psychol. Rep., 1967, 20, 124-126.

Essman, W. B. Facilitation of memory consolidation by chemically induced acceleration of RNA synthesis. Proc. XXIII Internat. Congr. Physiol. Sciences, 1965, 470.

Essman, W. B. Modification of neural RNA with amnesic agents and events. Proc. XVIII Int. Congr. Psychol., Moscow, 1966, 20, 65-67.

Essman, W. B. Alterations in memory consolidation as a function of the synthesis and turnover of neural 5-hydroxytryptamine. Seventh Int. Congr. Biochem., 1967, Tokyo.

1003546974

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#624 NOAKES

10035469975

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 29, 1967

Defer to next meeting

MEMORANDUM

TO: The committee comprising Dr. Jacobson, Chm., Dr. Loosli and Dr. Reimann.

FROM: Robert C. Hockett

SUBJECT: New grant application from John E. Noakes, Ph.D. - No. 624.

We enclose herewith a new grant application from Dr. John E. Noakes of the Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830.

Dr. Noakes made two or three visits to this office to discuss his past research and new plans, before submitting the application here enclosed.

In talking with him I had in mind the general attitude of the Board as expressed when the subject of Polonium-210 in tobacco first arose. The consensus, as I recall it, was that any realistic effort to define the possible health hazard due to polonium in tobacco must be part of the general problem of dose-response relationships in exposure to any alpha-emitting radioactive material. Since the Atomic Energy Commission is very deeply and extensively involved in such studies it seemed inadvisable for the Council to dabble with that problem on a small scale.

The applications from Werthamer (#595 and #596) were submitted to the Board in March 1967 to relieve the staff of responsibility in disposition. It was denied, (1) because of doubts expressed by Dr. Hasterlick about the proposed methodology, (2) because it proposed to study differences in lung burden of polonium in smokers vs non-smokers without any kind of provision for determining "how much is too much" in terms of hazards and (3) on the general grounds mentioned above.

The Noakes proposal is of quite a different character. The man impressed me as knowing what he was talking about and his institutional connection suggest that this should be the case. The proposed work is aimed at following up clues as to the origin of polonium-210 in tobacco with a possibility of demonstrating relatively simple and inexpensive means of reducing the content materially. The same study may also be pertinent to many other crop plants.

Whether or not polonium-210 is presently a hazard in tobacco, or whether this can easily be shown, it seemed to me that the industry might be interested in such means of minimizing this contamination, as a matter of public policy. I know that the companies have sponsored some work on polonium but do not know whether this has included anything substantially equivalent to what Noakes proposes here. Hence, I am sending a copy of this application to a member of the industry technical group for comment, and memorandum.

100546976

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

MEMORANDUM - CONT'D

Noakes included parts 2 and 3 of the proposal in a tentative manner. He is not a biologist and would not undertake systematic studies of animal exposures to smokes from tobaccos containing different known levels of polonium-210 "grown-in" without full consultation concerning methods of exposure and of systematic biochemical and pathological examination. He merely indicates that he would be glad to cooperate in setting up such studies and in providing the materials if this kind of follow-up should be wanted after the first phase. I think this question must be left in abeyance at present. We have been trying to develop better equipment and methods for exposing small animals chronically to whole, fresh, "normal" cigarette smoke and for measuring the actual exposures achieved. Such exposures have not so far given any clear or reproducible evidence of a direct carcinogenic effect of cigarette smoke on the lungs. Hence, we cannot judge at present how worthwhile it would be to try comparing the actions of three smokes with different polonium levels. Further experience gained the next year or so may help decide this question. Similar questions pertain to such biological test systems as that which Dr. Furst has been using, the Heston intravenous administration method that Dr. Homburger has been trying out, or to the mouse-skin painting method.

R. C. H.

P.S. I am enclosing a copy of a paper by C. W. Francis and G. Chesters of the University of Wisconsin entitled Radioactive Ingrowth of Polonium-210 in Tobacco Plants, which may anticipate Noakes to some degree. A copy has been sent to Noakes for his comment.

100354972

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

NO. 624

COMMITTEE:
Dr. Jacobson, Chm.
Dr. Loosli
Dr. Reimann

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.
633 THIRD AVENUE
New York, N. Y. 10017

Application for Research Grant

Date: July 11, 1967

1. Name of Investigator (s):

   John E. Noakes, Senior Scientist, Ph.D.

2. Institution & Address:

   Oak Ridge Associated Universities
   Post Office Box 117
   Oak Ridge, Tennessee 37830

3. Short Title of Project: "Po-210 in Tobacco"

4. Proposed Starting Date: September 1, 1967

5. Anticipated Duration of this Specific Study: Phase I (1 year), Phase II (2-3 years)

6. Brief Description of Objectives or Specific Aims:

   See Attachment

7. Give a Brief Statement of your Working Hypothesis:

   See Attachment

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

   See Attachment

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

   See Explanation on Page 2

10. Additional Requirements:

    A multichannel analyzer is needed for pulse height analysis in the radiometric determination of Ra-226, Pb-210 and Po-210. This instrumentation is available at the Special Training Division for short-term research projects, but teaching activities take priority. It is felt that the extensive use that would be made of this instrument in the proposed tobacco research would necessitate full-time uninterrupted use. In addition to the above equipment, one pH meter and two magnetic hot plates would be required. The present greenhouse would have to be enlarged and heat and lights installed in the greenhouse.

2003546978

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

11. Biographical sketches of all principal and professional personnel (append)

See Attachment

12. List of publications: (Five most recent as pertinent) (append)

See Attachment

9. Physical Facilities Available (continued from page 1)

Oak Ridge Associated Universities, until January 1, 1966, known as Oak Ridge Institute of Nuclear Studies, is a non-profit organization operated by 41 Southeastern U. S. universities serving as a prime contractor in educational, medical, and research activities for Oak Ridge Operations of the Atomic Energy Commission.

All facilities for the performance of the work proposed are owned by the Government and are operated by Oak Ridge Associated Universities for the Atomic Energy Commission under a cost-type management contract. Some of the necessary staff are also employed primarily for such AEC work. The AEC facilities may be used only for the performance of AEC programs, except as the AEC may approve the accomplishment of other work. The terms of our AEC contract contemplate the possibility of our performing such work when agreement that we do so has been reached with the AEC.

Should this proposal receive favorable action and the requested grant be made, agreement will be reached with the AEC regarding the manner in which the grant funds will be applied toward the cost of the work.

1003549679

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

13. Budget: (1st year)

	% Time	Amount
A. Personal Services:		
Professional		
Dr. John Noakes	30%	R
Technical		
Technician (Senior Grade)	70%	R
Sub-Total		R
B. Consumable Supplies		
Chemicals		200
Radioactive Standards		100
Glassware		200
Silverfoil		100
Platinum discs		150
Sub-Total		$ 750
C. Other Expenses		
Travel and Consultation		$ 750
Secretarial Help		300
Space and Facilities Use		3,100
Sub-Total		$ 4,150
D. Permanent Equipment		
128 Multichannel Analyzer		$ 6,500
Greenhouse Addition Including Heating & Lighting Equipment		2,500
pH Meter & Electrodes (1)		300
Magnetic Hotplates (2)		200
Sub-Total		$ 9,500
E. Overhead on A + B + C,		$ 4,400
Total		$30,900

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Perman. Exp.	Overhead	Total
Year 2	R	2,000	7,500	19,000	8,700	$61,200
Year 3		2,000	7,500	19,000	8,700	$61,200

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants are Made."

Signature John Noakes
Project Director

Signature William G. Pollard
Executive Director

Telephone 483-8411, Ext. 202

10035469B0

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
* Environmental Radiation Studies	AEC	$30,000/yr.	Thru 6/30/68
** Mechanisms of Radiation Injury Studies related to treatment of radiation injury Metals metabolism and medical radioisotope development, Therapy with radiation, Radioisotopes in diagnosis, Biologically important radioisotopic materials: Absorption, Whole Body Retention and Deposition in Specific Organs; NASA Study on radiation effects, Instrument development and methodology			

* Research by Special Training Division
**Research by Medical Division - while not directly related to the first phase of the proposed project, later work in this project will involve the Medical Division.

Pending

10035469 81

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

11a. Biographical sketches of all principal and professional personnel (append)

John E. Noakes

Birthplace: R R
Date of Birth: R
Education: B.S. - Champlain College - 1953
Graduate Study - Syracuse University
M. S. - Texas A & M University - 1959
Ph.D. - Texas A & M University - 1962

Member: Professional
American Chemistry Society
Honorary
Sigma XI

Academic Experience:

1962-Present: Senior Scientist, Special Training Division, Oak Ridge Associated Universities, senior lecturer with research in geochronology of marine sediments and environmental studies.

1961-1962 Assistant Professor (Research), Institute of Marine Science, University of Alaska; work concerned with establishment of a marine laboratory and research in clay minerology.

1960-1961 Research Scientist II, Texas A & M University; responsible for setting up and operating the carbon-14 dating laboratory using the liquid scintillation method.

1958-1960 Research Chemist, Texas A & M University; IGY Program, "Mohole" Investigation, and course instructor.

1957-1958 Research and teaching assistant and work in development of an analytical method for boron evaluation in marine waters; and investigation of long chain unsaturated fatty acids.

1955-six months Graduate Assistant at Syracuse University

Industrial Experience:

1003546982

1959-1962 Served as a consultant for toxicity work applied to industrial application.

1955-1957 Research Chemist and Junior Executive position, Clark-Cleveland Company. Work in product development in the field of plastics.

Personal contact with Battelle Memorial and Evans Research Centers with laboratory evaluation of their submitted data.

1953-1955 Soils Engineering - New York State, Soil Analyst

1965-Present Consultant - Picker Instrument Company

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Patents:

Low Temperature Conversion of Acetylene to Pure Benzene #424804

Publications:

"The Distribution of Boron and Boric Acid Complexes in the Sea," Master Thesis, Texas A & M University, 1959

"Boron-Boric Acid Complexes in Sea Water," presented at the International Union of Geodesy and Geophysics, Helsinski, Finland, 1960 and Noakes, J. E. and D W. Hood, Jour. of Deep Sea Research, 8, 121-129, 1961.

"C^{14}/C^{12} Ratios of the Organic and Inorganic Carbon Fraction of Waters of the Caribbean and Gulf of Mexico," Final Report A & M, Project 235, N.S.F. Grant-610232, 15 February 1961.

"Carbon Dating by Liquid Scintillation," Noakes, J. E. and D. W. Hood, presented at the Oak Ridge Special Course, "Nuclear Methods as Applied to Oceanography," November, 1961.

"Benzene Synthesis Acids C^{14} Dating," Chemical and Engineering News, October 9, 1961.

"Conversion of Carbon Dioxide to Benzene for Liquid Scintillation Counting," Noakes, J. E., A. S. Isbell, and D. W. Hood, Trans. Amer. Geophys. Union, 42, No. 2, 226, 1961.

"Univ. of Texas Radiocarbon Dates I," Stipp, J. J., E. M. Davis, J. E. Noakes, and T. E. Hoover, Amer. Jour. of Sci. Radiocarbon, Suppl. 4, 1962.

"Natural Radiocarbon Measurements by Liquid Scintillation Counting," Dissertation, Texas A & M University, 1962.

"Low Temp Benzene Synthesis for Carbon-14 Dating," Noakes, J. E., A. F. Isbell, J. J. Stipp and D. W. Hood, Geochemica et Cosmochimica Acta, 27, No. 7, 797-804, 1963.

"Texas A & M University Radiocarbon Dates I," Noakes, J. E., J. J. Stipp, and D. W. Hood, Amer. Jour. of Sci. Radiocarbon Suppl., 6, 189-193, 1964.

"Geochronology of the Gulf of Mexico, Part I," Rona, E., L. K. Akers, J. E. Noakes, I. R. Supernaw, Progress in Oceanography, Vol. 3, Perganon Press, 1965.

10035469B3

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Publications (Cont'd)

"A Chemical Study of an Ambient Temperature Catalytic Benzene Synthesis Used in Radiocarbon Dating, Noakes, J. E., S. M. Kim, G. A. Thomas, and L. K. Akers, ORINS Publication #46, November, 1964.

"Cobalt Molybdate Catalyst for Ambient Temperature Synthesis of Benzene for Liquid Scintillation Radiocarbon Dating," Noakes, J. E., S. M. Kim and L. K. Akers, ORINS-50, April, 1965.

"Chemical and Counting Advances in Liquid Scintillation Counting," J. E. Noakes, S. M. Kim and J. J. Stipp, Pullman Conference - June 7-11, 1965, Paper presented and published in proceedings. U.S.A.E.C. Conf.-650652

"Electrodeposition of Actinides and Lanthanides," Kim, S. M., John E. Noakes, L. K. Akers, W. W. Miller, ORINS-48, December 15, 1965.

"Electrodeposition Method for Counting Alpha and Beta Emitters," Kim, S. M., J. E. Noakes, and W. W. Miller, Nucleonics, 24, #3, March, 1966.

"Anomalies in the Th^{230}/Th^{232} Activity Ratio in Some Mississippi River Sediments, Noakes, J. E. and I. R. Supernaw, (presented at AGU Meeting in April, 1965. Journal Geophysical Research (in press)

"Recent Improvements in Benzene Chemistry for Radiocarbon Dating," J.E. Noakes, S. M. Kim and L. K. Akers, Geochimica and Cosmochimica Acta (in press)

"Oak Ridge Institute of Nuclear Studies Radiocarbon Dates I," Noakes, J. E., S. M. Kim and L. K. Akers, Jour. of Sci. Radiocarbon Suppl. (in press)

"The Mass of a Neutron: A Student Exercise", H. E. Banta and J. E. Noakes, American Journal of Physics (accepted for publication)

"Uranium Content of Gulf of Mexico Sea Water", J. E. Noakes, S. M. Kim and L. K. Akers, Presented at American Geophysical Union, April, 1967 (to be published)

"A New Method for Thorium Analysis from Marine Sediment," S. M. Kim, J. E. Noakes, Presented at American Geophysical Union, April, 1967 (to be published)

10035469984

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

APPLICATION FOR RESEARCH GRANT

(6) Objectives of Proposed Po-210 Tobacco Research

The first phase of the program would be a geochemical study of the sources of Po-210 which might be available to tobacco plants and the gross mechanism by which Po-210 could enter the plant system. The second phase would be a medical study to determine if and at what concentration levels Po-210 in biological systems could be considered to be carcinogenic.

Specific Aims on a Yearly Basis

(a) Identification of parent radioactive source material most responsible for the occurrence of Po-210 in tobacco (Ra-226, Rn-222, Po-210).

(b) Mechanism by which Po-210 parent finds its way into the plant system (air-plant interface, soil-root interface, etc.).

(c) Establish conditions for decreasing Po-210 content in tobacco plants grown in a natural environment (phosphate fertilizer, soil pH control, etc.).

(d) Verify controlled conditions for growing tobacco with low and high concentrations of Po-210 for medical studies.

Second Year

(a) Bulk amounts of tobacco grown with high and ultra low Po-210 content under conditions established from first year study.

(b) Bulk tobacco of high and low Po-210 content analyzed for trace metal content, tars, nicotine, etc., to be used as standardized reference tobacco sources for biological studies.

(c) Consultation and preliminary evaluation of most suitable biological specimens to be used in medical studies.

(d) Perfection of methods and equipment for introducing tobacco smoke into biological specimens and analytical methods to be employed in the evaluation of results. (c and d - Current tobacco research projects to be evaluated.)

Third Year

(a) Medical studies of biological specimens subjected to high and low concentration of Po-210 tobacco smoke.

(b) Pathological and chemical analysis to be carried out on sacrificed animals to determine Po-210 content in various organs any evidence of alteration in cell development.

1003546985

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Fourth Year

(a) Continuation of medical investigation looking into specific aspects of third year study which indicates further investigation is warranted.

(7) Working Hypothesis

Radford and Hunt (Science 1964) were first to publish the finding of high concentration of Po-210 found in the respiratory and pulmonary systems of tobacco-smoking individuals. The radiation dose rate in bronchial epithelium for long duration smokers (2 packs a day for 25 years) was calculated to be as high as 200 REM. This value far exceeds the maximum dose of 1.1 REM to an entire lung as recommended by the International Council for Radiation Protection (ICRP). They concluded that the dose was sufficient to cause alteration in cell development and could be an important source for initiating bronchial cancer in smokers. More recent studies have reported Po-210 found in the liver, kidneys, blood, etc., of tobacco-smoking people which has led to supposition that it should be a prime suspect as a cancer forming agent.

Skrable (Science 1964) and others have taken exception to this work and formulated mathematical models to substantiate their statement. Because of the rather limited experimental and analytical data available for the biological half-life of Po-210 and its alpha radiation damage to various organs, no mathematical model can be constructively applied.

The occurrence of Po-210 in tobacco has also raised the question as to the source and mechanism by which this radionuclide finds its way into the plant. Studies by Gregory (Science 1965) on the Po-210 content in tobacco obtained from all parts of the world show a wide variation. U. S. tobaccos rank among the highest in Po-210 content with values of 1-0.5 pc/g of tobacco.

Berger (Science 1965) has attributed the Po-210 content of tobacco as resulting from the assimilation of Rn-222 of gas at the plant-atmosphere interface. Atmospheric Rn-222 concentration is between 50-200 pc/m^3 and originates from the decay of Ra-226 in ground materials. Berger's work has concentrated on showing that the major portion of the Po-210 uptake in tobacco occurs after harvest and during the curing period.

Tso (Science 1966) has attempted to refute this work by showing that a more plausible explanation for Po-210 in tobacco is in plant root uptake of Pb-210 from the soil. He demonstrated in his studies that tobacco plants subjected to high concentrations of Po-210 for a short growth period can contain Po-210 as high as 150 pc/g of tobacco.

1003546986

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Po-210 content of tobacco has been of ORAU staff interest since the first reports appeared in the literature. Speculation was that one source of Po-210 in tobacco could be the Florida phosphate rock used as a phosphorous source for essentially all fertilizers in the United States. This phosphate rock is reported to contain 0.01-0.05% uranium and would be expected to have in secular equilibrium, the radioactive daughter Po-210. Radiometric analysis of the major constituents of commercial tobacco fertilizers have revealed that Po-210 occurs in the phosphate rock fraction in concentrations of 20-25 pc/g. The yearly application of high phosphate fertilizer to American tobacco growing soils may well be the prime reason for the high Po-210 found in U. S. tobaccos.

Further studies have been carried out at ORAU to determine if Po-210 free tobacco could be grown in a controlled environment. Tobacco plants grown hydroponically in nutrient solution free of Po-210 or its precursors have been found to contain Po-210 in concentrations of <.01 pc/g of tobacco. These studies and those of Tso, clearly show that Po-210 content in tobacco can be regulated to desired amounts under controlled experimental conditions. These studies also indicate that similar controlled experiments could be devised to determine the source and mechanism for uptake of Po-210 into tobacco plants.

The ability to produce tobacco with regulated amounts of Po-210 also offers potential application to biological studies. Introduction of controlled amounts of Po-210 in smoke to biological specimens would facilitate quantitative evaluation of such parameters as Po-210 uptake, body distribution and retention times. It would also enable comparison of biological specimens possessing low and high Po-210 content for carcinogenic evaluation.

(8) Experimental Design

The experimental procedures proposed for accomplishing the first year's goals set forth in section 6 of this proposal can best be described as three sets of experiments which will be concurrently carried out.

I. Radioactive materials responsible for Po-210 in tobacco plants and gross mechanism of plant uptake.

Three groups of tobacco plants will be greenhouse raised. One group of plants will be grown in quartz sand free of Po-210 or any parent radioactive material (photo 1). The second plant group will be grown in typical virgin Tennessee tobacco soil. The third group of plants will be grown in similar soil, but will have phosphate fertilizer added to them which contains appreciable amounts of Po-210. All plant groups will be nourished with nutrient solution made with reagent grade chemicals free of any Po-210 or parent radioactive material.

1003546987

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

All tobacco plants from the three groups will be harvested at maturity. The quartz sand grown tobacco plants should show the lowest Po-210 content of all tobaccos and reflect only the uptake of parent Rn-222 at the atmospheric-plant interphase during time of growing and curing. The tobacco Po-210 content from atmospheric Rn-222 contribution during curing will be evaluated by preferential curing methods.

The Po-210 content of tobacco plants grown in soil with nutrient added will reflect the soil uptake and the Rn-222 contribution. The Po-210 furnished by the soil can be calculated by subtraction of the Po-210 contribution from Rn-222.

The Po-210 content of tobacco plants grown in soil with phosphate fertilizer added will be a composite value of Rn-222 uptake, plus soil contribution and fertilizer contribution. Since the Po-210 soil and Rn-222 contribution will be known, the fertilizer Po-210 contribution to the tobacco can be calculated.

It should be pointed out that the Po-210 soil contribution to the tobacco plant at the time of harvest can be composite value of the direct non-supported Po-210 uptake from the soil and parent grow-in. Ra-226, Pb-210 and Po-210 analysis of the mature tobacco at harvest and at later times will permit calculation of the decay of the non-supported Po-210 and the grow in of the Po-210 parent contribution. These analyses will also enable identification of the Po-210 parent which is entering the plant-root system and soley responsible for the Po-210 content in aged smoking tobaccos.

II. Conditions for decreasing Po-210 content in soil grown tobacco plants.

Three groups of tobacco plants will be grown in Tennessee soil under greenhouse conditions. Each plant will receive a known amount of radioactive Po-210 in equilibrium with its daughter Po-210 and nutrient solution of controlled pH.

The first group of plants will be raised with nutrient solution of pH5. The second group will be raised with nutrient solution of pH7. The third group will have nutrient solution of pH7 with added sulfate ions.

Plants will be grown to maturity and analyzed at harvest time for Po-210 and Po-210 content. The first group of plants will represent uptake under acid soil conditions which should reflect less tendency of the soil to hold the available Pb-210 and Po-210. The second plant group will represent uptake of Pb-210 and Po-210 from the added radioactive spike and the normal soil contribution. Plants grown in the third group under pH7 soil conditions with excess available sulfate ions should indicate the degree of formation of insoluble Pb-210 sulfate as compared to available Pb-210 for plant uptake.

1003546988

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

The presence of high concentrations of sulphate ions in commercial fertilizers is due to the sulfuric acid treatment of phosphate rock during fertilizer manufacturing. The possibility of the formation of insoluble $PbSO_4$ in soil at raised pH should therefore be considered.

Soil pH is known to be a prime factor in affecting plant trace metal uptake. If the tobacco uptake of Pb-210 and Po-210 is found to be altered by soil pH, it may be one explanation for the wide variations in Po-210 content found in tobaccos grown in all parts of the world. It may also reveal advantages to be gained by incorporation of additives to fertilizer or soils to better control soil pH.

III. Pb-210 and Po-210 tobacco uptake studies to establish conditions for growing tobaccos of high Po-210 content.

Three groups of tobacco plants will be grown in quartz sand under greenhouse conditions. All plants will be fed nutrient solution for their entire growth period. Each group of plants, early in its development will be subjected to a designated amount of radioactive Pb-210 in equilibrium with its daughter Po-210. Radioactivity administered to the first group will be 1x, the second group 10x and the third group 100x, where x = μ curie levels.

All tobacco plants will be raised to maturity and analyzed at time of harvest for Pb-210 and Po-210 content. These results will establish the upper levels of Po-210 content which can be obtained in tobacco. Low level Po-210 tobacco data will be supplied from series I experiments. A plot of the Po-210 tobacco uptake with regard to available Pb-210 and Po-210, will be used to establish predictable conditions for growing tobacco of desired Po-210 content to be used in the medical studies.

ANALYTICAL METHODS

I. Tobacco trace metals, tar and nicotine content

Tobacco grown under experimental conditions (photo 2) will be analyzed for trace metals, tar and nicotine content. Similar analyses of commercial tobaccos will be conducted for comparative pruposes. Trace metal analysis will be carried out using non-destructive slow and fast neutron activation analysis. The tar and nicotine analyses will be conducted by various methods.

Bates

1003546989

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

II. Radioactive Analyses of Ra-226, Pb-210 and Po-210

Radiometric analysis of Ra-226, Pb-210 and Po-210 are standard methods which are routinely taught and practiced at the Special Training Division of ORAU. The tobacco and fertilizer analyses for these three isotopes are carried out by digestion and dissolution with strong oxidizing agents. Ra-226, and Pb-210 are separated using ion exchange chromatography and electroplated onto platinum discs by applied potential. Po-210 is spontaneously electroplated onto silver discs by its own redox potential. The quantitative yields for the chemical separation and electrodeposition of each of these three isotopes has been determined by radioactive tracers.

Alpha counting is carried out with solid state detectors or ion chambers (photo 3) of measured counting efficiency. Alpha spectroscopy (photo 4) using pulse height analysis is employed for quantitative measurement of these isotopes.

1003546990

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

AEC FACILITIES AND EQUIPMENT REQUIRED TO CARRY OUT PROPOSED RESEARCH PROJECT,

"Po-210 in Tobacco"

Facilities & Equipment	Equipment Acquisition Cost	Equipment Acquisition Date	% Time Usage
1. Laboratory, 10' x 10', equipped with the following:			100%
a. Sink with hot and cold running water			
b. Distilled water facility			
c. Bench top, 3'x10'			
d. Fume hood with electrical outlets and water			
e. Storage cabinets and shelves			
f. Desk			
2. Greenhouse, approximately 108 square feet			100% for approximately four months
3. Liquid Scintillation Counter, prop. #8798	$13,825	August, 1965	20%
4. Ion Chamber prop. #7149	$ 669	June, 1959	30%

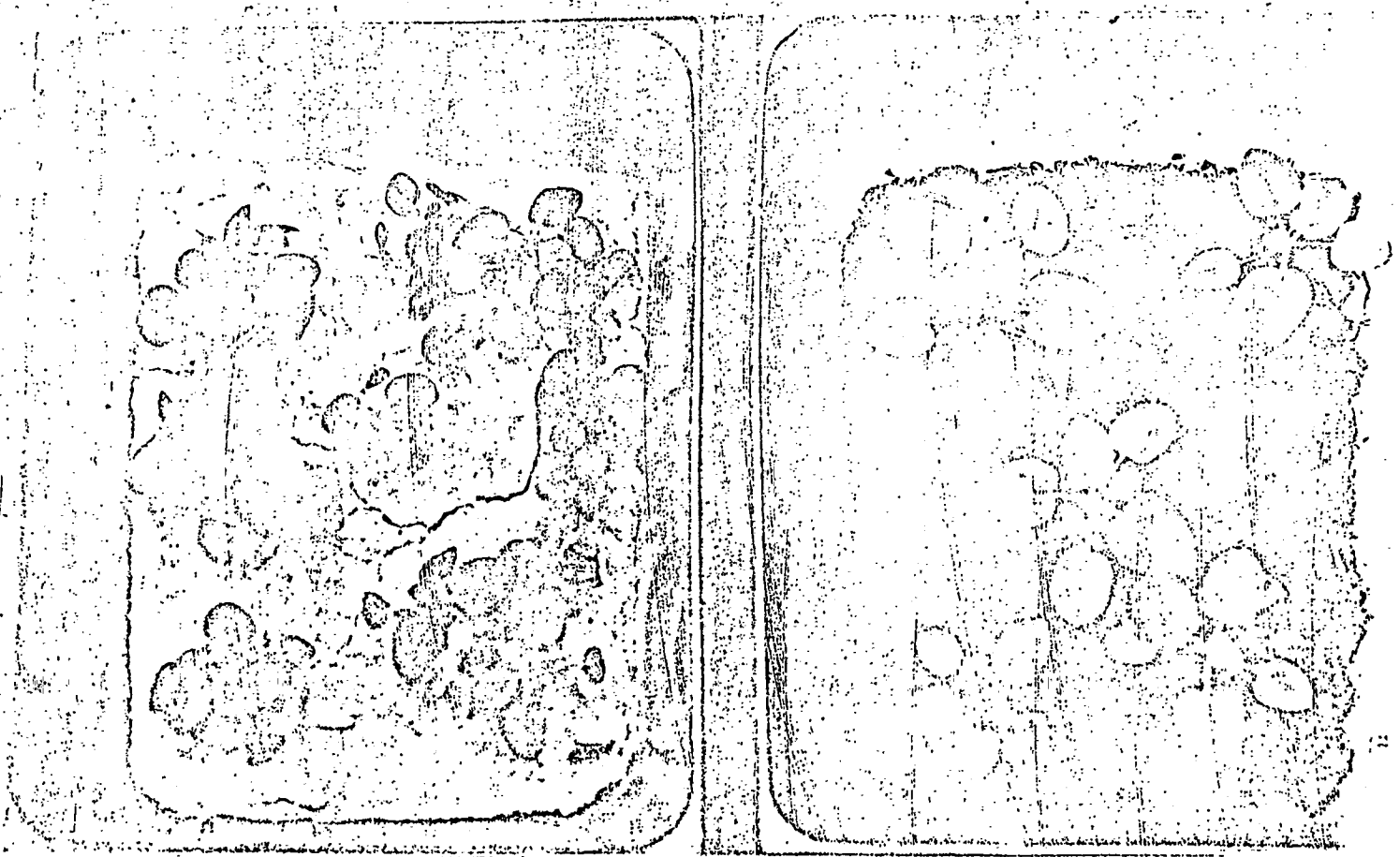
10035469 91

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Photo 1
Greenhouse Grown Tobacco Plants

Quartz Sand

Tennessee Soil



100354992

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

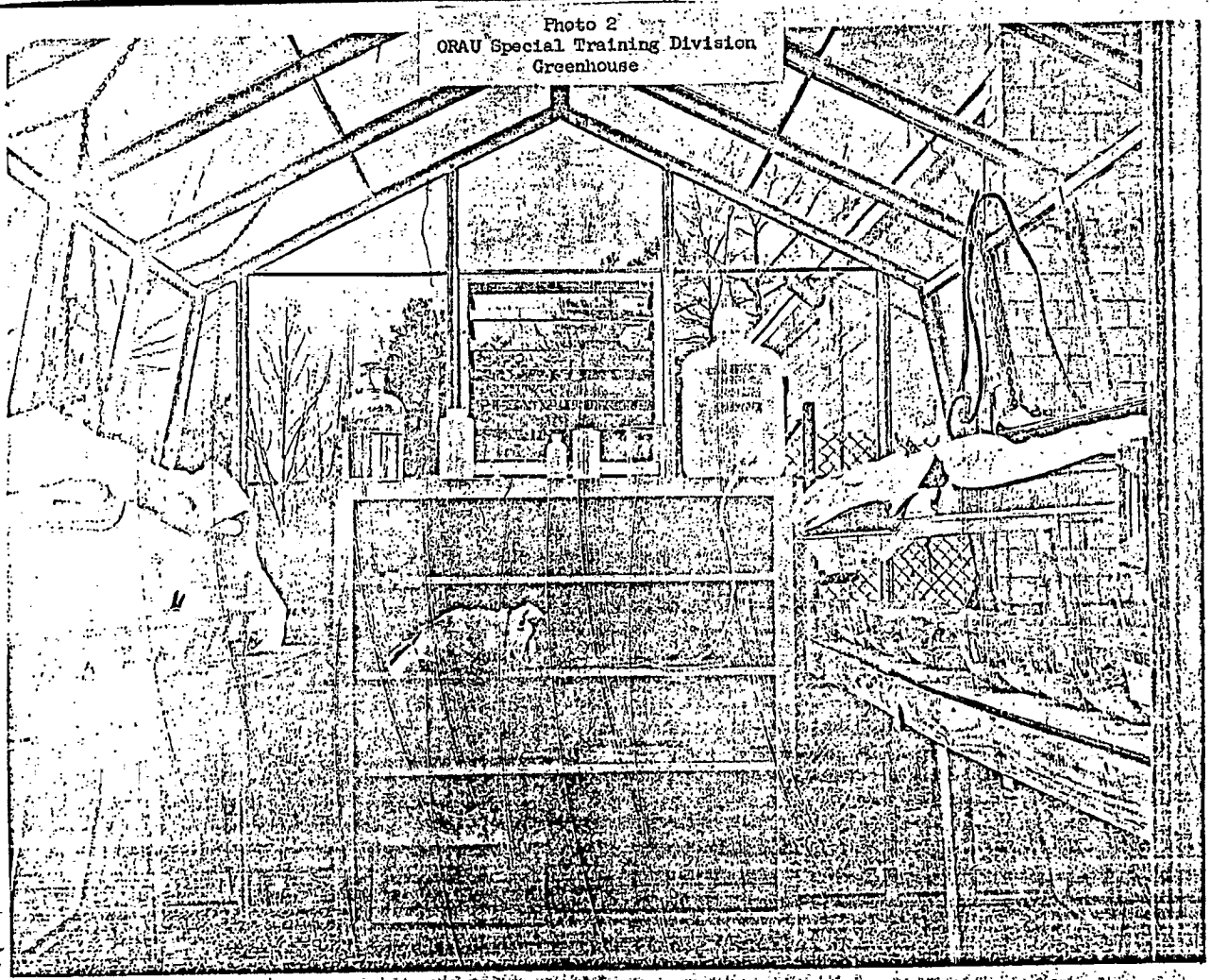


Photo 2
ORAU Special Training Division
Greenhouse

1003546993

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003546994

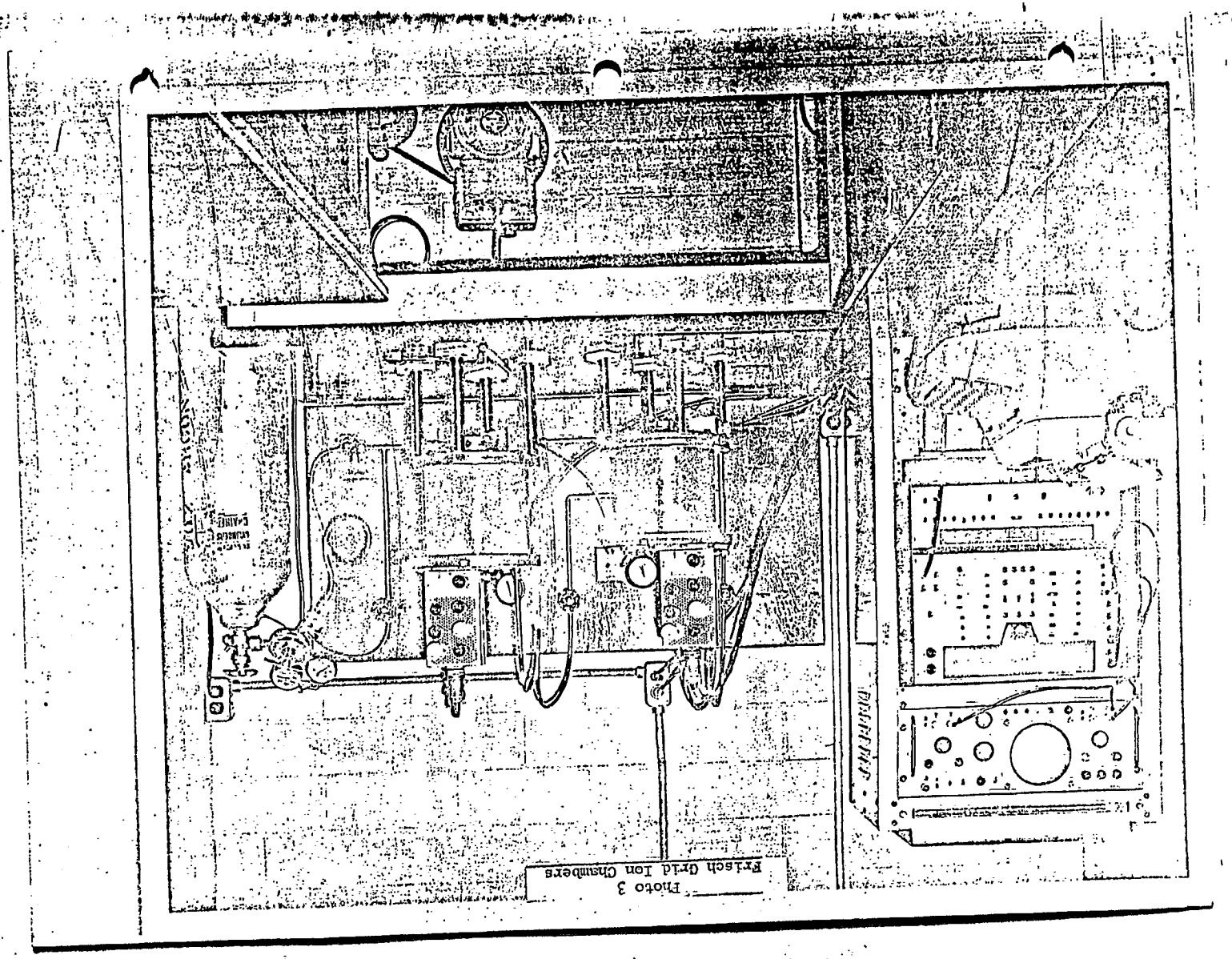
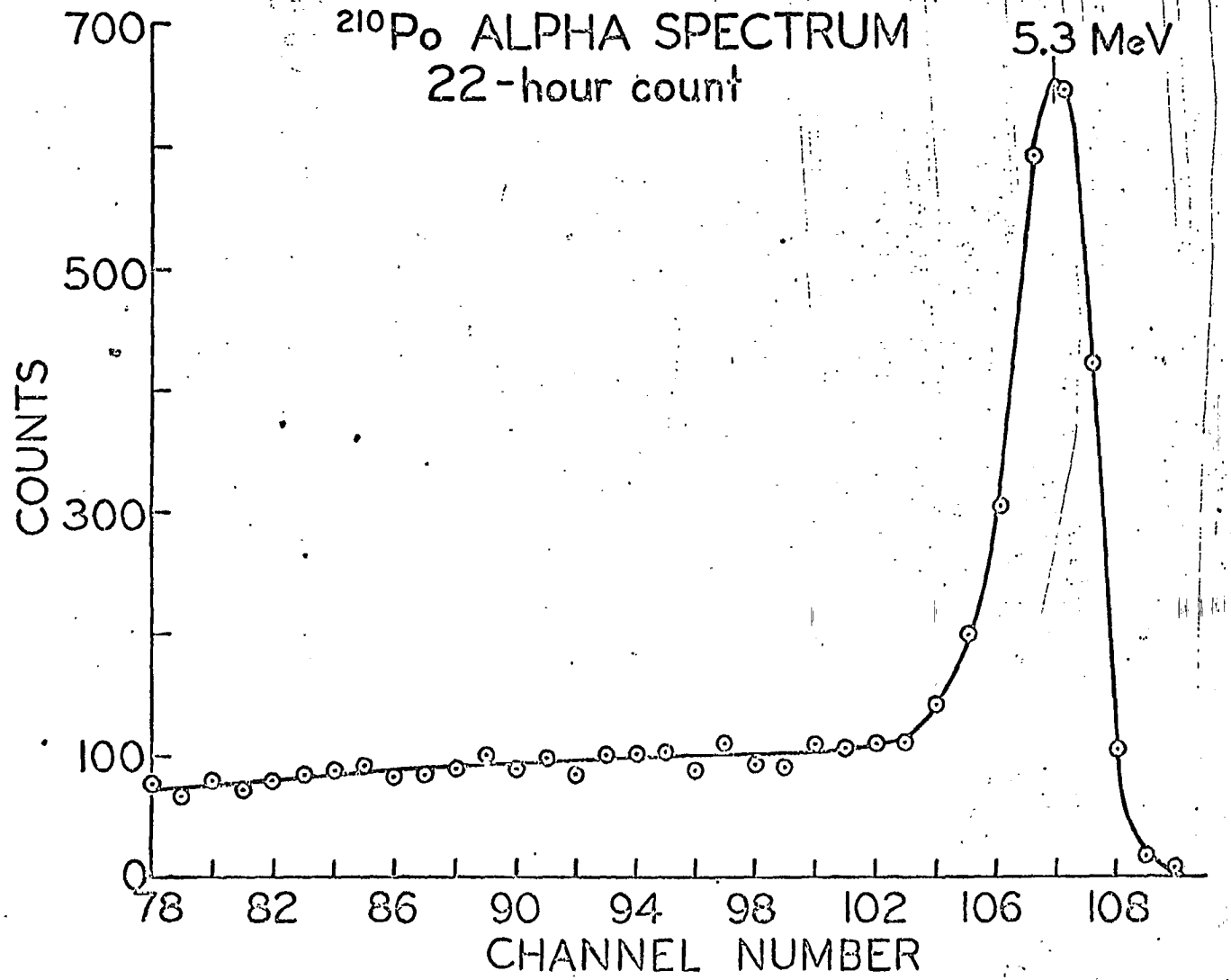


Photo 3
Prisch Grid Ion Chambers

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

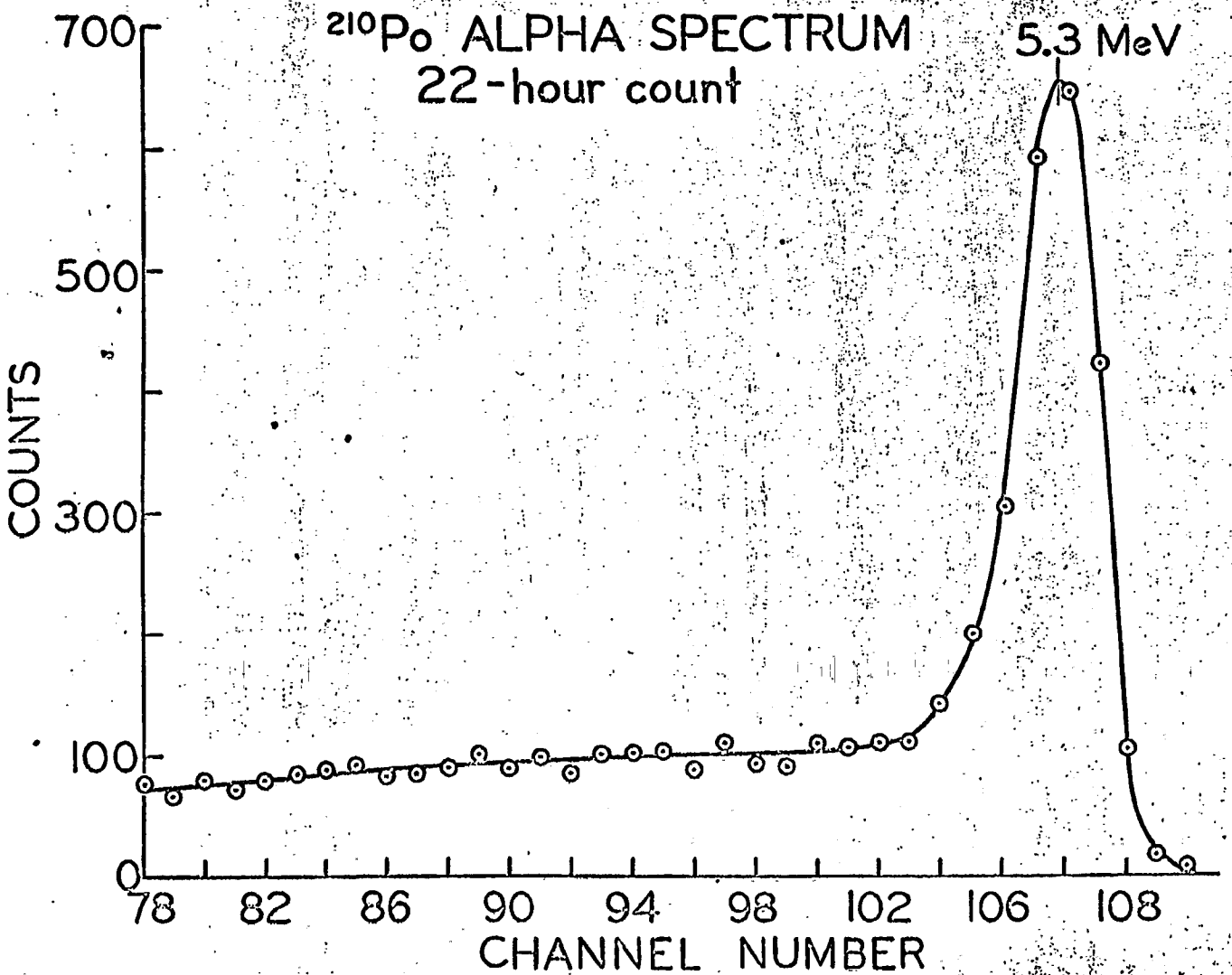
Photo 4



1003546995A

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Photo 4



1003546995

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#625 BROWN

1003546996

Source: https://www.industrydocuments.ucsf.edu/docs/ptdn0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

July 25, 1967

Rejected

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Sommers.

FROM: Robert C. Hockett

SUBJECT: New application from Harry Darrow Brown, Ph.D. - No. 625.

We enclose herewith a new application from Dr. Harry Darrow Brown of the University of Texas, Galveston, Texas.

The application includes a supplement on "Nicotine Effects Upon Cardiac Membranes" and is accompanied by six reprints (one an abridgement) of studies by the author on ATPase as listed below.

1. Harry Darrow Brown. A characterization of the ouabain sensitivity of heart microsomal ATPase. Biochem. Biophys. Acta, 120, 162, (1966).

2. Harry Darrow Brown, S.K. Chattopadhyay, and A. Patel. Sarcoplasmic Reticulum ATPase On a Solid Support. Biochem. Biophys. Res. Comm. 25, 304, (1966).

3. Harry Darrow Brown. Azasteroids and Heart Adenosine Triphosphatase. Biochem. Pharmacal., 15, 2007, (1966).

4. Harry Darrow Brown, Swaraj K. Chattopadhyay, Anil B. Patel. Properties of Butanol-Extracted and Detergent-Solubilized Membrane ATPase. Arch. Biochem. Biophysics, 120, 222, (1967).

5. H. D. Brown, S.K. Chattopadhyay, A. Patel and R. H. Rigdon. Glycoside Effect Upon Membrane Enzymes of Erythrocytes and Muscle in Duck Myopathy. Experientia.

6. H. D. Brown (?) (Authors not shown). Abridgement of the Manuscript "Erythrocyte Abnormality in Human Myopathy."

R. C. H.

1003549997

Source: https://www.industrydocuments.ucsf.edu/docs/ptdn0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY No. 625

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Sommers

Application For Research Grant

Date: July 7, 1967

1. Name of Investigator(s): (include Title and Degrees) Harry Darrow Brown, Ph.D.
Assistant Professor of Biochemistry

2. Institution & Address: The University of Texas Medical Branch at Galveston, Texas

3. Short Title of Project: Nicotine effect upon cardiac membrane enzymes

4. Proposed Starting Date: October 1st, 1967

5. Anticipated Duration of this Specific Study: 2 years

6. Brief Descripton of Objectives or Specific Aims:

It has been reported that the administration of nicotine elevates serum cholesterol level in experimental animals. We propose here to extend this observation to a study of the primary and secondary effects of nicotine upon enzymes associated with subcellular membranes of cardiac tissues.

The specific aim of the project is to test the thesis that nicotine in changing the nature of the lipid pool, from which cellular membranes are synthesized, may alter the function of those enzymes which are bound, in the native state, to membrane portions of cardiac muscle.

7. Give a brief statement of your working hypothesis.

It is known that nicotine intervenes in the biosynthesis of lipids and this in turn may change the nature of the lipid pool available for and ultimately incorporated into heart muscle sarcoplasmic reticular and plasma membranes. From this, we may predict that the changed lipid composition of the membrane system will result in a changed function of those enzyme systems which are part of the membrane structural network. It is primarily the ion transport, membrane adenosine triphosphatase and the hormonally-controlled adenyl cyclase systems which we propose to study. The thesis of membrane change will be further examined by a consideration of serum enzymes whose levels in serum are relateable to cellular changes. We hypothesize that the reported change in lipid composition which follows nicotine administration affects the ion transport system and so is an event preliminary to cardiomyopathy.

1003546998

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

A. Biological material.

Rodents and rabbits will be used as experimental animals. Continued injection of nicotine following a dose rate based upon Kershbaum, et al. (Am. Heart Jour. 69: 206, 1965) will be undertaken.

B. Observation of serum lipids.

During the course of administration of nicotine, the level of serum lipids will be assayed by thin-layer chromatography using silicic acid. Additional experimental procedures will be designed upon the basis of the changed pattern of serum lipids so that the differences observed in consequence of nicotine administration may be intensified in another group of experimental animals by the injection of lipids in ratios relative to those found in the prime experimental group.

It has been our recent findings that the administration of a 20, 25 diazacholesterol to experimental animals greatly affects the activity pattern of transport ATPase of red blood cells and muscle membranes.

As in natural myopathic states, erythrocytes and muscle tissue are altered

(continued page 2A)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

(see page 2A)

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append).

(see page 2B)

12. List of publications: (Five most recent as pertinent) (append)

(see page 2C)

10035469999

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

by the administration of diazacholesterol. This phenomenon we attribute to a change in the lipid components of the membranes to which the protein enzyme is bound. The changed activity pattern (response to metals and to the cardiac steroid membrane) represents an extremely sensitive indicator of membrane subcellular organization.

C. Enzyme assay.

The systems chosen for primary study are known to be associated with membrane structures of the cell. The transport ATPase is probably the best documented model for transport phenomena and its vectorial role in cell function closely relates to membrane structure. The adenyl cyclase system appears to be part of a structure-related complex of catalysts acting upon ATP as substrate. The pivotal position of adenyl cyclase, whose catalysis is facilitated by epinephrine and whose product in turn controls the phosphorolysis step of glycolysis, may be a key to interpretation of energetic cataolism and its biological control. These two membrane related enzymes will serve as indicators of drug (nicotine) induced changes.

Aldolase and creatine phosphokinase elevation in serum are widely used indicators of tissue degeneration. These will be assayed and correlated with the hypothesized cardiomyopathy.

Blood will be collected in a haemolyzing solution. The blood haemolyzate will be centrifuged at 20,000 x g for 20 minutes. The supernatant discarded and the pellet washed and recentrifuged in the Tris buffer. The final pellet obtained will be collected and stored as the erythrocyte ghost preparation, and used as the source of ATPase activity. Muscles will be macerated and sarcoplasmic reticulum isolated by a differential centrifugation procedure in sucrose. Heart muscle membrane preparations prepared in much the same way will be assayed for adenyl cyclase activity by ultra violet analysis following ion exchange chromatography. These methods and those for the assay of aldolase and creatine phosphokinase activity in serum are presently in use in our laboratory. The ATPase will be assayed by measurement of inorganic phosphate; aldolase by the Sibley-Lehninger method and creatine phosphokinase by measurement of phosphoryl group transfer.

D. Analysis of data.

Findings will be interpreted in terms of the effect of nicotine administration upon changes in membrane enzyme and in serum enzyme levels (presumably related to linkage from cells). This deviation will be correlated with induced changes in the metabolic lipid pool and all factors considered as a function of possible cardiomyopathy.

9. Facilities available.

Three laboratories are in use by the principal investigator. Many major analytic tools are available. Specific holdings directly available to this project are: Beckman B flame spectrophotometer, International HR 1 refrigerated centrifuge, Beckman L ultracentrifuge, RSCo fraction collector, ISCO fraction collector, deionizing column, water baths, Zeromatic pH meter, Cahn electro-balance, Roller-Smith balance, Beckman DB recording spectrophotometer, refrigerators, freezers, necessary minor laboratory devices and ware. Held by a colleague in an adjoining laboratory and available to the project are F and M gas chromatographs, Cary 14 spectrophotometer, and P-E infrared spectrophotometer.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Additional common facilities held by the Department in another building, available to this project, include a 2000 square foot laboratory, animal rooms, refrigeration equipment, Mettler balance, free electrophoresis unit, analytic ultracentrifuge and recording spectrophotometer. Also the resources of the Institution include electron microscope facilities, infrared spectrophotometer, Beckman amino acid analyzer, scintillation counter, and generally, most of the instrumentation and facilities appurtaining to the laboratory investigation. A unique dual differential microcalorimeter is in use in this laboratory and appropriate to aspects of the proposed study.

11. Biographical sketches of all principal and professional personnel.

Harry Darrow Brown

R R

m., 3 c.

Ph.D.	1957 Columbia University. Faculty of Pure Science
A.M.	1952 same
B.S.	1950 Long Island University
1964-	Assistant Professor, Department of Biochemistry and Nutrition, University of Texas Medical Branch, Galveston, Texas.
1964-	Collaborator, United States Dept. of Agriculture.
1963-1964	Chemist (Biochemist), USDA, New Orleans.
1961-1963	Associate Professor, Southern Illinois University, Edwardsville.
1957-1961	Assistant Professor, Loyola University, New Orleans.
1954-1956	Lecturer, Hunter College, New York.
Occasional	Editor: Macmillan, Acad. Press, Schol. Lib.

Anil B. Patel

R R

M.S.*	University of Cincinnati
M.Sc.	1961 University of Bombay
B.Sc.	1953 University of Bombay
1966-	Research Associate, Department of Biochemistry, The University of Texas Medical Branch, Galveston, Texas.
1963-1965	Research assistant in organic chemistry, University of Cincinnati.
1962-1963	Teaching assistant in chemistry, University of Cincinnati.

* Residence requirements complete; thesis in preparation.

10035472001

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

12. List of five recent publications pertinent to the proposed research.

Brown, H.D., Chattopadhyay, S.K., and Patel, A.B., (1967) Erythrocyte abnormality in human myopathy. Ms. submitted.

Brown, H.D., Chattopadhyay, S.K., Patel, A., and Rigdon, R.H., (1967) Glycoside effect upon membrane enzymes of erythrocytes and muscle in duck myopathy. Experientia, in press.

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1966) Sarcoplasmic-reticulum ATPase upon a solid support. Bioch. Bioph. Res. Comm. 25 (3): 304-308.

Brown, H.D., (1966) Azasteroids and heart adenosine triphosphatase. Biochem. Pharmacol., 15: 2007-2011.

Brown, H.D., (1966) A characterization of the ouabain sensitivity of heart microsomal ATPase. Bioch. Bioph. Acta 120: 162-165.

10035472002

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)		% time	Amount
Professional			
Principal investigator			
Research associate, Anil Patel		100	R
Technical			
Secretarial services		15	
Technical assistant		50	
OASI, WCI			
		Sub-Total	R
B. Consumable Supplies (list by categories)			
Chemicals	380.		
Chart paper, stationery	50.		
Hardware, small tools	75.		
Glassware	160.		
Silica curvettes (4)	80.		
		Sub-Total	745.
C. Other Expenses (itemize)			
Craftsman's services	70.		
Communications, xerox	80.		
Travel (to consult with colleagues and to present results)	450.		
Publication costs	70.		
Animals and animal care	800.		
		Sub-Total	1470.
D. Permanent Equipment (itemize)			
Tanks and accessories for thin-layer chromatography	350.		
			350.
E. Overhead (15% of A+B+C)		1591.	1591.
		Total	12548.

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	745.	1470.	none	1654.	12681.
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Harry Darrow Brown
Director of Project 713 SO 5 - 1107
Telephone

Signature Warren G Harding
Business Officer of the Institution
Telephone

1003547003

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current				
	No support for this project is available from other sources.			
Pending				

10035470004

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Harry Darrow Brown
R R
m., 3 c.

Ph.D.	1957 Columbia University. Faculty of Pure Science
A.M.	1952 same
B.S.	1950 Long Island University
1964-	Assistant Professor, Department of Biochemistry and Nutrition, University of Texas Medical Branch, Galveston, Texas.
1964-	Collaborator, United States Dept. of Agriculture.
1963-1964	Chemist (Biochemist), USDA, New Orleans.
1961-1963	Associate Professor, Southern Illinois University, Edwardsville.
1957-1961	Assistant Professor, Loyola University, New Orleans.
1954-1956	Lecturer, Hunter College, New York.
1950-1953	Teaching Assistant, Columbia University.
Occasional	Editor: Macmillan, Acad. Press, Schol. Lib.

Present areas of scholarship:

The enzymes of vectorial (transport) metabolism, relationship of subcellular structure to enzyme function, microcalorimetry applied to biochemical events.

Manuscripts in preparation:

Membrane ATPase and muscle disease; enzyme studies in the myopathic mouse; routine calorimetric medical analysis; a book 'Biochemical Microcalorimetry' being written together with a group of distinguished collaborators, under my editorship, will be published by Academic Press.

Recent bibliography:

Brown, H.D., Chattopadhyay, and Patel, A.B. (1967) Erythrocyte abnormality in human myopathy. Ms. submitted.

Patel, A.B., Brown, H.D. (1967) On the mechanism of trypsin esterase activity. Ms. submitted.

Brown, H.D., Rigdon, R.H., Chattopadhyay, S.K., and Patel, A. (1967) Enzyme studies in the myopathic duck. Ms. submitted.

10035470005

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Brown, H.D., Chattopadhyay, S.K., and Patel, A.B. (1967) Erythrocyte ghost ATPase. Ms. submitted.

Patel, A.B., and Brown, H.D. (1967) Selective deamination of nucleosides by 2,4 dinitrophenyl hydrazine. Nature, 214: 402.

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1967) Properties of butanol-extracted and detergent-solubilized membrane ATPase. Arch. Biochem. Biophys. 120: 222.

Schuster, C.F., Shannon, G.R., and Brown, H.D. (1967) Temperature control of collected fractions. Ms. submitted.

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1967) The apparent multiplicity of membrane ATPase activities. Biophys. J. Supp. VII, Abst. WA8.

Chattopadhyay, S.K., Patel, A., Rigdon, R.H., and Brown, H.D. (1967) Transport enzyme abnormality in myopathy. Proc. 7th Intl. Biochem. Congress (abst.) in press.

Brown, H.D., Chattopadhyay, S.K., Patel, A., and Rigdon, R.H. (1967) Glycoside effect upon membrane enzymes of erythrocytes and muscle in duck myopathy. Experientia, in press.

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1966) Sarcoplasmic-reticulum ATPase upon a solid support. Bioch. Bioph. Res. Comm. 25 (3): 304-308.

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1966) Restructuring, by linkage to insoluble matrices, of enzymes solubilized from cell particles. J. Cell Biol., 31 (2): 17A (abst.).

Brown, H.D., Chattopadhyay, S.K., and Patel, A. (1966) Characteristics of an ATPase in membrane particles, solubilized, and linked to a cellulose matrix. Enzymologia, in press.

Brown, H.D. (1966) Simultaneous 3-element microcalorimetry of experimental and control enzyme reactions. Proc. Second Intl. Biophysics Congress. (abst. #692).

Brown, H.D., Patel, A., and Chattopadhyay, S.K. (1967) Cellulose-matrix supported biological substances. Ms. submitted.

Brown, H.D. (1966) Azasteroids and heart adenosine triphosphatase. Biochem. Pharmacol., 15: 2007-2011.

Brown, H.D., Jackson, R.T., and Waitzman, M.B. (1967) Ciliary process ATPase: azasteroid and erythrophleum alkaloid inhibition. Life Sciences, in press.

Chattopadhyay, S.K., and Brown, H.D. (1966) A new detergent useable in enzyme solubilization. Texas Jour. Sci., 18 (3): 324.

Brown, H.D., Patel, A., and Chattopadhyay, S.K. (1966) The properties of apyrase upon a solid support. Plant Physiol., 41 (supp.) lxvi (abst.).

Huffman, Gary and Brown, H.D. (1966) Heart membrane ATPase-model system for inotropic activity. Texas Reports of Biol. and Med. 24 (3): 512 (abst.).

Brown, H.D. (1966) Sampler for automated chemical analysis. Chemist-Analyst. 55 (3): 88-89.

Brown, H.D. (1966) A characterization of the ouabain sensitivity of heart microsomal ATPase. Bioch. Bioph. Acta 120: 162-165.

Brown, H.D. (1966) Membrane preparation and steroid-azasteroid sensitivity of transport ATPase. In, Snell, F., Membranes and transport phenomena. Biophy. Soc., St. Louis.

Brown, H.D. (1966) Membrane preparation and steroid azasteroid sensitivity of transport ATPase. Biophys. Jour. VI Supp.: 135 (abst.).

Brown, H.D., Neucere, N.J., Altschul, A.M., and Evans, W.J. (1965) Activity patterns of purified ATPase from Arachis. Life Sciences 4: 1439-1447.

1003547006

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

Brown, H.D., Evans, W.J., and Altschul, A.M. (1965) Applications of calorimetry: schema for the continuous observation of the movement of glucose across a biological membrane. Bioch. Biophys. Acta 94 (1): 302-304.

Brown, H.D., Evans, W.J., and Altschul, A.M. (1964) Analysis by differential calorimetry of ATPase activity in potato apyrase and red-blood-cell ghosts. Life Sciences 3: 1487-1492.

Altschul, A.M., McCourtney, E.J., Evans, W.J., Carney, W.B., and Brown, H.D. (1964) Properties of a preparative polyacrylamide gel electrophoresis column as they affect yield and column stability. ACS abst. Chicago, Sept. 1964.

Altschul, A.M., Evans, W.J., Carney, W.B., McCourtney, E.J., and Brown, H.D. (1964) Some aspects of preparative electrophoresis on polyacrylamide gel: application to bovine serum albumin. Life Sciences 3: 611-615.

Brown, H.D., and Altschul, A.M. (1964) Glycoside-sensitive ATPase from Arachis hypogaea. Bioch. Bioph. Res. Comm. 15: 479-483.

Brown, H.D., Altschul, A.M., Evans, W.J., and Neucere, N.J. (1964) Characterization of a purified ATPase from Arachis hypogaea with special reference to the relationship of activity to conformation. Plant Physiol. 39 (supp) lxi-lxii (abst.).

Brown, H.D., Evans, W.J., and Altschul, A.M. (1964) Study of ATPase by differential calorimetry. Fed. Proc. 23 (2): 175 (abst.).

Brown, H.D., Jackson, R.T., and DuPuy, H.J. (1964) Transport of sugar in Allium: Effects of inhibitors and ethylene. Nature 202 (4933): 722-723.

Jackson, R.T., and Brown, H.D. (1963) Cation and glucose transport in onion epidermis. J. Cell. and Comp. Physiol. 61 (3): 215-222.

Brown, H.D. (Ed.) (1963) Cell Interface Reactions. Schol. Lib., N. Y.

Memberships:

REDACTED

REDACTED

REDACTED

10035420007

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Supplement to

Application for Research Grant

The Council for Tobacco Research

NICOTINE EFFECT UPON CARDIAC MEMBRANE ENZYMES

Harry Darrow Brown
The University of Texas Medical Branch at Galveston
Galveston, Texas 77550

713 - SO5 - 1107

1003547008

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Diazacholesterol effect upon membrne ATPase

HARRY DARROW BROWN, SWARAJ K. CHATTOPADHYAY and ANIL B. PATEL
Galveston, Texas.

Conditions resembling myopathy have been induced in experimental animals by the administration of steroids. Response of animals to these compounds has been reported as characteristically a moderate to severe muscular weakness bearing a similarity to myotonia.[1,2] Winer, et al.[3] using 20, 25 diazacholesterol in man and in the goat reported that the drug induced a delayed muscle fiber relaxation after contraction, indistinguishable from naturally occurring myotonia.

We have found in man and in the duck[4,5] that certain myopathic states can be correlated with an altered response to the cardiac glycoside, ouabain, of membrane-bound ATPase from erythrocyte ghosts and from the sarcoplasmic reticulum of skeletal muscle. In the present study we undertook a prolonged administration of 20, 25 diazacholesterol to rabbits while monitoring the response of isolated erythrocyte-ghosts ATPase to ouabain. Effect of the drug course upon muscle membrane ATPase preparations was assayed at the termination of the treatment period.

Materials and methods

Fifteen male rabbits (6-7 lbs.) were used in three groups. Each animal from Group I was treated with a 5 mg daily dose of 20, 25 diazacholesterol in 1 ml of water intramuscularly. Similarly, animals from Group II were given higher doses of the drug which were progressively increased from 10 mg to 50 mg in 1 ml of water daily. The control Group III was maintained upon a daily administration of 1 ml of distilled water.

From the Biochemistry Department, The University of Texas Medical Branch at Galveston, Texas.

1003542009

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Nine to ten ml of blood was collected in a glass tube containing 10 ml haemolyzing solution (Tris buffer, 0.002 M, pH 7.4, with 0.005 M Na_2EDTA) from each animal. Blood samples were collected before the treatment and after 7, 8, 11 and 13 days of administration. Skeletal muscle samples were collected after 16 days of drug therapy. Each time, the blood haemolyzate was centrifuged at 20,000 x g for 20 minutes. The supernatant was discarded and the pellet washed and recentrifuged 4 to 5 times in the same Tris buffer, and 2 mM NaCl. Before each wash the pellet was homogenized in a power driven Teflon-pestle homogenizer for 5 minutes to ensure complete disruption of blood cells. The reddish-white pellet obtained in final centrifugation was collected and stored at -10°C as the erythrocyte ghost preparation, and used as the source of ATPase activity.

Muscles were macerated in 10 volumes of 0.1 M cold Tris buffer, pH 7.2, with 0.25 M sucrose preliminary to separation of the membrane fractions by centrifugation. The slurry was first centrifuged 600 x g for 20 minutes. The pellet was rejected and the supernatant dialyzed against the same Tris-sucrose buffer with 5 mM Na_2EDTA for 4 hours. Afterward the dialysates were centrifuged 10,000 x g and 20,000 x g for 30 minutes, and each time the pellets were discarded. This supernatant was then centrifuged in 10 ml tubes at 80,000 x g for 30 minutes and again 100,000 x g for 70 minutes. The 100,000 x g pellet was resuspended in 2 ml Tris-sucrose buffer, pH 7.2, as the enzymatically active fraction (sarcoplasmic reticulum).

ATPase activity was measured as inorganic phosphate evolved in reaction mixtures containing 0.1 ml of enzyme preparation (ghost or membrane fraction), 0.8 ml substrate (0.1 M Tris-sucrose buffer, pH 7.2, with Na_2ATP [0.3 mg/ml of reaction mixture] together with 0.001 M $MgCl_2$, 0.002 M KCl, 0.001 M NaCl), 0.1 ml water or of inhibitor in water. Multiple reactions were run so that they might be taken as samples in sequence to allow consideration of the extent of reaction as a function of time. Reaction mixtures were incubated at 42°. After incubation, 0.1 ml of 50% trichloroacetic acid was added and the mixture was centrifuged at 600 x g for 6 minutes. The supernatant was assayed for inorganic phosphate. Protein was determined in sample aliquots of the enzyme preparation.

100354710

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Results

Blood was drawn from each of the animals before the initiation of the administration of diazacholesterol. At this time erythrocyte-ghost ATPase activity was inhibited by 10^{-4} M ouabain in the incubation mixture, without exception. In the first experimental group, animals receiving 5 mg daily doses, the erythrocyte adenosine triphosphatase activity was stimulated by ouabain, rather than inhibited as were the control preparations. This was true also of preparations from blood of rabbits in experimental Group II, which had received larger doses. These data are presented in Table I.

Muscular weakness began the 4th day of drug administration and became progressively severe. This was accompanied by an apparent muscle atrophy.

Figure 1 A is a graphic representation of the result of a series of experiments in which ATPase activity of ghosts was plotted as a function of time and compared (dotted line) with a similar series, using ghosts prepared from the same animal, in which the catalytic activity was inhibited by 10^{-4} M ouabain. Identical experiments using preparations from animals which had been carried on courses of administration of 20, 25 diazacholesterol are presented by Figures 1 B and 1 C. The effect of ouabain here was to stimulate rather than to inhibit the ATPase activity. The results of the drug course upon the 100,000 x g muscle fraction (sarcoplasmic reticulum) ATPase was similar to that upon the red-blood-cell ATPase; enzyme activity of control animals was inhibited while that of the experimental animals was stimulated. Data obtained in representative series of experiments is presented in Figure 2.

Discussion

The characteristic response of membrane ATPase systems to ouabain is an inhibition of the rate of catalytic hydrolysis of ATP. Enzymatic activity of ghost preparations and a muscle membrane fraction from untreated rabbits was inhibited by ouabain. Sustained treatment of animals with 20, 25 diazacholesterol changed the character of the ATPase activity such that a stimulation of the catalytic rate was effected by the presence of ouabain in the incubation mixture. We have

1003547011

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

suggested[6, 7] that the ATPase response to ouabain is a reflection of the conformation of the enzyme which it derives in part from its association with the membrane.

Myopathic changes in skeletal muscle following the administration of corticosteroids has been discussed by Awad, et al.[2] In their experimental work the possibility of neural induction of the observed myopathy was eliminated and they concluded that steroid myopathy is a primary muscular disorder.

The use of 20, 25 diazacholesterol as a cholesterol lowering agent was discontinued in medical practice when it became known that the site of action was at the point of conversion of desmosterol to cholesterol resulting in the accumulation of desmosterol. The present results which show an effect upon membrane related ion transport enzyme ($Na^+ + K^+$ -ATPase) may provide an element of support to the thesis that diazacholesterol causes myotonia by affecting the muscle membrane system. Such an effect could conceivably follow a change in the lipid pool from which the membrane elements in turn are synthesized. It is known that the ATPase activity is affected by changes in the lipid components of the membrane.[8] The interrelationship of lipid pool to functional membrane is a matter which requires, and is subject to, direct experimental test.

Rabbits in the experimental groups visibly showed indications of changes in muscle tone which may relate to the observations of Winer, et al.[3] that the diazacholesterol is capable of inducing myotonia. The transport ATPase response to ouabain which we have found in erythrocytes from myotonic patients[4] is paralleled by the present experimental results in the diazacholesterol-treated rabbit. These findings accord with the common supposition that myotonia involves aberrant transport phenomena and appears to indicate that diazacholesterol administration directly affects the membrane transport system.

1003547012

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Table I. Effects of ouabain upon ATPase activity* (μmoles Pi/mg Protein/min) of erythrocyte ghosts. (Group I animals received 5 mg diazacholesterol/day; Group II progressively increasing doses 10-50 mg/day; Group III, controls.)

Identification	Drug administered	Activity		
		Control	Ouabain 10^{-4} M	% stimulation inhibition
Group I.				
1	35 mg	0.20	0.27	+35
	65 mg	0.22	0.26	+18
2	35 mg	0.16	0.17	+ 6
	65 mg	0.19	0.21	+10
3	40 mg	0.48	0.47	- 2
	65 mg	0.26	0.39	+50
4	40 mg	0.19	0.19	+ 0
	65 mg	0.40	0.59	+47
5	65 mg	0.23	0.35	+52
Group II.				
6	140 mg	0.21	0.25	+19
	360 mg	0.39	0.36	- 7
7	140 mg	0.13	0.15	+15
	360 mg	0.32	0.36	+12
8	180 mg	0.29	0.46	+58
	360 mg	0.28	0.55	+96
9	360 mg	0.24	0.49	+104
10	310 mg	0.15	0.24	+60
	360 mg	0.21	0.32	+52
Group III.				
11		0.14	0.11	-21
12		0.16	0.11	-25
13		0.14	0.06	-57
15		0.15	0.12	-20
17		0.44	0.28	-36

* Upon the basis of 40 minute incubation.

100354713

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

REFERENCES

1. Faludi, Georgina, Mills, Lewis C., and Chayes, Zev W.: Effect of steroids on muscle, Acta Endocrinologica 45: 68-78, 1964.

2. Awad, Essam A., Swaiman, Kenneth F., and Kittke, Frederic J.: Changes in the structure, innervation, electromyographic patterns and enzymes of skeletal muscle resulting from experimental treatment with triamcinolone, Arch. Physical Med. and Rehabilitation 46: 297, 1965.

3. Winer, Nathaniel, Martt, Jack M., Somers, John E., Wolcott, Lester, Dale, Homer E., and Burns, Thomas W.: Induced myotonia in man and goat, J. of Laboratory and Clin. Med. 66: 758-769, 1965.

4. Brown, H.D., Chattopadhyay, S.K., and Patel, A.B. Erythrocyte abnormality in human myopathy. Ms. submitted.

5. Brown, H.D., Chattopadhyay, S.K., Patel, A., and Rigdon, R.H.: Glycoside effect upon membrane enzymes of erythrocytes and muscle in duck myopathy. Experientia, in press.

6. Brown, H.D.: A characterization of the ouabain sensitivity of heart microsomal ATPase. Bioch. Bioph. Acta 120: 162-165, 1966.

7. Brown, H.D., Neucere, N.J., Altschul, A.M., and Evans, W.J.: Activity patterns of purified ATPase from Arachis, Life Sciences 4: 1439-1447, 1965.

8. Schatzmann, H.J.: Lipoprotein nature of red cell adenosine triphosphatase, Nature 196: 677, 1962.

1003547014

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

(Abstract)

ATPase activity of erythrocyte ghost preparations and of a muscle membrane fraction (sarcoplasmic reticulum) from untreated rabbits was inhibited by the cardiac glycoside, ouabain. Sustained treatment of animals with 20,25 diazacholesterol, reported by Winer et al., (this Journal, 66: 758, 1965) to induce myotonia, changed the character of the ATPase activity of identical preparations such that a stimulation of the catalytic rate was effected by the presence of ouabain in the incubation mixture. We have earlier suggested that the ATPase response to ouabain is a reflection of the conformation of the enzyme which it derives in part from its association with the membrane. The present findings accord with the common supposition that myotonia involves aberrant transport phenomena and appears to indicate that diazacholesterol administration directly affects the membrane transport system.

1003543045

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#627. BELLER

1003547016

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

ARCHIVE VARIANCE SHEET

THE NUMBER (RANGE) 1003547017 IS

( ) MISSING

( ) MISSING IN ALL SETS

( ) MISSING IN CLOSED AND REVIEW SETS

( ) DELETED

( ) CHANGED TO _____

(X) DELETED AND CHANGED TO 1003546995 A

( ) NOT USED

( ) OTHER

DATE OF CHANGE

CLOSED SET 3-1-85

REVIEW SET 5-1-85

WAREHOUSE SET _____

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 18, 1967

MEMORANDUM

TO: The committee comprising Dr. Bing, Chm., Dr. Cattell and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New grant application from Fritz K. Beller, MD., Sc.D. - No. 627.

We enclose herewith a new grant application from Dr. Fritz K. Beller, New York University School of Medicine, New York, New York.

The author has developed a method for producing disseminated intravascular coagulation in rabbits by the infusion of endotoxin. Using this model he proposed to determine the effects of various factors including tobacco combustion products. Details of the technique for administering the combustion products of tobacco are not revealed.

R. C. H.

In support of this application we enclose seven reprints:

1. Beller, F.K. and Graeff, H.: Deposition of Glomerular Fibrin in the Rabbit after Infusion with Endotoxin. Nature, in press. (July 15-copy)

2. Beller, F.K., Mitchell, P. and Gorstein, F.: Fibrin Deposition in the Rabbit Kidney Produced by Protease Inhibitors. Thrombos. Diathes. Haemorrh. 17:427,(1967)

3. Beller, F.K., Debrovner, Ch.H. and Douglas, G.W.,: Potentiation of the Lethal Effect of Endotoxin by Heterologous Plasma. J. exper. Med. 118:245,(1963)

4. Beller, F.K., Douglas, G.W. and Epstein, MD.: The Fibrinolytic Enzyme System in Newborn. Amer. J. Obstetr. Gynec. 96:977, (1966)

5. Beller, F.K. and Porges, R.: Blood Coagulation and Fibrinolytic Enzyme Studies during Cyclical and Continuous Application of Progestational Agents. Amer. J. Obstetr. Gynec. 97:448,(1967)

6. Maki, M. and F.K.Beller: Comparative Studies of Fibrinolytic Inhibitors in Vitro. Thrombos. Diathes. Haemorrh. 16:668,(1966)

7. Beller, F.K., Goessner, W. and Herrschlein, Hans J.: Tissue Activator of the Fibrinolytic System in Placental Tissue. Obstetr. Gynec. 20:117,(1962).

1003547018

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Bing
Dr. Cattell
Dr. Jacobson
Dr. Lynch

Reject

Application For Research Grant

Date:

1. Name of Investigator(s): (include Title and Degrees)

Fritz K. Beller, M.D., Med. Sc. D., F.A.C.O.G., Professor

2. Institution & Address:

New York University School of Medicine
550 First Avenue, New York, N.Y. 10016

3. Short Title of Project:

The influence of tobacco smoking on intravascular proteolysis

4. Proposed Starting Date: October 1, 1967

5. Anticipated Duration of this Specific Study: 3 years

6. Brief Descripton of Objectives or Specific Aims:

The inference of tobacco combustion products, including nicotine and carbon monoxide on blood coagulation will be studied in an experimental model in which intravascular coagulation is initiated under carefully controlled conditions.

It has been shown recently in this laboratory that a single continuous intravenous infusion of a sublethal dose of endotoxin in rabbits produces glomerular fibrin deposition and renal cortical necrosis. Although the histopathology of fibrin deposition is identical to that of the generalized Shwartzman reaction, this experimental model differs from that of the generalized Shwartzman reaction where two injections of a sublethal dose of endotoxin are administered 24 hours apart. The difficulty of interpreting the overlapping effects of two doses of endotoxin is avoided by the continuous intravenous infusion, which is also able to initiate fibrin deposition as a dose related phenomenon. The pathophysiology of disseminated fibrin deposition has not been fully elucidated. However, endotoxin has several distinct, direct and indirect effects on blood coagulation. Platelets fall in a linear fashion, followed by a subsequent decrease of the "consumable" coagulation factors including fibrinogen. Leukocytes decrease initially but increase after 4-5 hours during the course of the infusion. We have observed that the complement titer falls in concert with hemolysis and fibrin deposition. The thrombi in the lung, liver and heart developed in an irregular pattern in the early phases, that is 2-3 hours following the initiation of the infusion; glomerular fibrin deposition is not seen until approximately 6 hours after the start of endotoxin administration. Renal shutdown is related to glomerular fibrin deposition. The development of glomerular capillary necrosis is apparently dependent on the following:

(continued on next page)

7. Give a Brief Statement of your Working Hypothesis:

Our initial observations indicate that the infusion of endotoxin in rabbits produces a reliable and reproducible method for disseminated intravascular

(continued on next page)

1003547019

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Question 6, continued:

1. the amount of glomerular involved
2. the reactivity of the kidney and its ability to lyse fibrin
3. the size of the dead vessels involved

The study proposed is designed to examine the effect of:

1. the gaseous combustion product of tobacco in its entirety
2. nicotine
3. carbon monoxide

In both acute and chronic experiments utilizing animals pretreated with a continuous intravenous infusion of endotoxin.

Question 7, continued:

coagulation as well as the initiation of the fibrinolytic system. Such a model permits the investigation of a variety of substances which might either protect the animal on the one hand or potentiate the dilatarious effects of endotoxin on the other. If the combustion products of tobacco effect the coagulation system per se, they manifest in several ways:

a) in the lowering of the total dose of endotoxin required for fibrin deposition.
b) the earlier initiation of coagulation or fibrin deposition.
c) the failure of lysis of fibrin or delay of lysis of fibrin.
d) any combination of these effects.

In addition, an effect not obvious in the untreated animal may become manifest.

1003547020

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

While the basis of the investigation will be the infusion of rabbits with a continuous dose of endotoxin (20-80 mg/Kg/hour) for periods of 6-14 hours, several distinct experimental plans will be followed.

Plan 1: In the study of the acute effects of tobacco combustion products, animals will be infused with endotoxin and exposed to nicotine, carbon monoxide and tobacco smoke. Either simultaneously or immediately prior to the infusion of endotoxin animals will be studied with respect to:

a) the dose range of endotoxin in controls as compared to treated animals,
b) the time lag required for glomerular fibrin deposition, and
c) any change in the parameters to be studied by serial blood sampling.

In the second plan endotoxin will be infused for a period of 10-14 hours, following which one kidney will be surgically removed (control). The animals will then be exposed to tobacco combustion products for an additional 10-14 hours and sacrificed. The kidney so obtained will be compared to that of the control kidney with respect to the deposition of fibrin in glomerular capillaries.

In plan 3 the animals will be exposed to tobacco combustion products over extended periods of time and subsequently infused with endotoxin. The dose range and parameters of coagulation will be studied in comparison with that of the controls.

(continued on next page)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

A fully equipped laboratory for the study of blood coagulation and the fibrinolytic enzyme system, with standardized techniques as well as histological and histopathological techniques is available. This laboratory is located in Bellevue Hospital as part of the Department of Obstetrics and Gynecology.

10. Additional Requirements:

Smoking machine (at proposal of Dr. Kuschner, Department of Pathology)

11. Biographical sketches of all principal and professional personnel (append)

check appended pages

12. List of publications: (Five most recent as pertinent) (append)

check appended pages

10035427021

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Question 8, continued:

Methods:

Among the methods employed will be frozen and permanent tissue sections utilizing conventional histochemical techniques for the demonstration of fibrin. Among the organs studied will be the liver, lung, heart, pituitary as well as kidney. Immunofluorescent methods employing anti rabbit fibrinogen produced in the rat will be utilized to substantiate the specificity of fibrin deposition. Histochemical identification of tissue activator will be studied utilizing the fibrin films and suitable tissue sections (Todd).

The coagulation system will be studied from the following points:

1- platelet enumeration (phase contrast)
2- fibrinogen (Ratnoff and Menzie), factor V (on phase method) and factor VIII levels (TGT):
3- thromboelastogram (Hartert)

Study of the fibrinolytic enzyme system: a) euglobulin lysis time, b) plasminogen, c) plasmin inhibitor and kinase inhibitor (Johnson et al.) fibrin plates heated and unheated (Astrup).

Biological parameters: a) arterial pressure, b) osmolarity, c) urinary output, d) body temperature, e) blood pH and pCO_2, f) leucocytes, g) hemolysis.

Question 11:

Principle Investigator: Fritz K. Beller, M.D., age 43. MD Marburg 1948, Internship and residency in Obstetrics & Gynecology, University of Giessen 1948-1954. Max Plank Institute of Radiobiology (Trainee German Research Council 1954), Dozent Obs & Gyn, University of Giessen (Med. Sci. D. 1955) Assistant and Associate Professor, University of Tuebingen 1956-1961. Professor of Obstetrics & Gynecology, Tuebingen 1961.

Visiting Associate Professor, NYU School of Medicine, Dept. Ob-Gyn, 1961-1963. Associate Professor, Dept. of Ob-Gyn 1963-1967. Professor of Ob-Gyn, NYU School of Medicine, September 1, 1967. Career Scientist of the Health Research Council of the City of New York 1961 to present.

German Specialty Board, 1957. Diplomate American Board of Obstetrics and Gynecology 1967. State Board New York 1963.

Societies: FACOG, FRSM (London) Soc. Gynecol. Investigation, Amer. Soc. Exp. Pathol., Intern. College Pathol., New York Acad. Sci., NYU Chapter XS.
U.S. Citizenship; 1966.

Co-Investigator: Fred Gorstein, M.D., age 37, MD NYU School of Medicine 1955. Intern Bellevue Hospital 1956-1957. Assistant Resident Pathology, Bellevue Hospital 1957-1960.
Fellowships: PHS Training Fellow Pathology 1957-1960, PHS Post doctoral Fellow Pathology 1960-1961. Career Scientist of the Health Research Council of the City of New York 1963 - present. Instructor Pathology, NYU School of Medicine 1960-1963. Assistant Professor 1963-1967. Associate Professor, Sept. 1,1967.
Diplomate American Board of Pathology 1961,1967.
Societies: N.Y. Acad. Sci., Harvey Soc., Citizen; U.S.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Question 11: continued:

Senior technician: Peter Mitchell, age 25, B.S. Cornell University 1963, Worked in this laboratory since 1963. Continued school at NYU at night. M.S. expected 1968. Citizen; U.S.

Technician: Helge Bornhausen; age 33, German Gymnasium. School for Medical Technicians Mainz 1955-1957. Joined this laboratory in 1966. Citizen; Germany. Immigrant Visa.

Question 12:

1) Beller,F.K. and Graeff,H.: Deposition of glomerular fibrin in the rabbit after infusion with endotoxin. Nature, in press.

2) Beller,F.K., Mitchell,P. and Gorstein,F.: Fibrin deposition in the rabbit kidney produced by protease inhibitors. Thrombos. Diathes. Haemorrh. 17:427, 1967.

3) Beller, F.K., Debrovner,Ch.H. and Douglas,G.W.: Potentiation of the lethal effect of endotoxin by heterologous plasma. J. exper. Med. 118:245, 1963.

4) Beller,F.K., Douglas,G.W. and Epstein,M.D.: The fibrinolytic enzyme system in newbornes. Amer. J. Obstetr. Gynec. 96:977, 1966.

5) Beller,F.K. and Porges,R.: Blood coagulation and fibrinolytic enzyme studies during cyclical and continuous application of progestational agents. Amer. J. Obstetr. Gynec. 97:448, 1967.

1003547023

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names):		
Professional		
Fritz K. Beller, M.D.	50%	R
Fred Gorstein, M.D. (co-investigator)	50%	
Fringe Benefits		
Technical		
1 technician	100%	
1 senior technician	100%	
Fringe Benefits		
Sub-Total		R
B. Consumable Supplies (list by categories)		
Glassware		
Laboratory reagents (chemicals immune sera, endotoxins, etc.)		
		1500
Sub-Total		1 500
C. Other Expenses (itemize)		
Laboratory animals		
		3000
Sub-Total		3000
D. Permanent Equipment (itemize)		
Thromboelastogram		2500
Water bath		200
Deep freeze unit		300
2 ovens		600
slide file cabinet		150
		3750
E. Overhead (15% of A+B+C)		2601
Total		26391

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Totall
Year 2	16167	1500	3000	500	3100	24267
Year 3	16800	1500	3000	500	3195	24995

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature _____
Director of Project

OR9-3200 ext.2732
Telephone

Signature _____
Business Officer of the Institution

George E. Armstrong, M.D.
Director

Telephone

10035470Z4

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

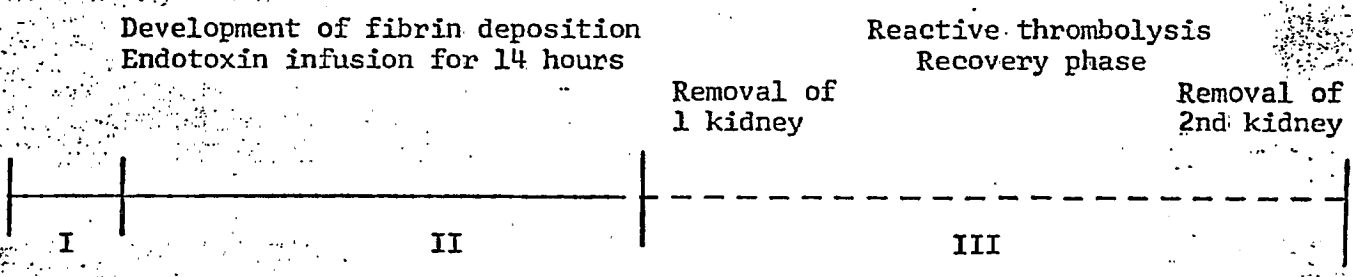
	Title of Project	Source	Amount	Duration
Current	NIH Trainee, Research Grant 10%		---	---
Pending	Biochemical Events of Endotoxemia	United States Army	60,000	3 years

1003547025

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Additional information to Grant request; Dr.Beller.

The principle parameter for the evaluation of smoke and smoke substances are primarily not parameters of the coagulation and fibrinolytic enzyme system in peripheral blood. They will be assayed but are of secondary interest. The primary interest will be focused on biological parameters in ~~XXXX~~ organ systems, especially the kidney after disseminated coagulation is produced by endotoxin infusion. The experimental design is best appreciated from the following scheme.



Experimental design:

I. Pretreatment phase: Smoke or smoke substances will be applied to animals acute or chronically before endotoxin infusion is induced.

Parameters: 1) Assaying the concentration of endotoxin needed to produce fibrin deposition with and without smoke substances. 2) Measuring the time of phase II until fibrin deposition occurs. Assays and methods: 1) histologic sections and 2) histochemical sections of the kidney, pituitary, liver and lung. The kidney removed in different time lags in between 4-14 hours is particularly suitable. 3) Renal excretion study. 4) ~~XXXX~~ Urine osmolarity. 5) Venous and arterial pH. 6) Leucocytes. 7) Coagulation assays.

1003547026

II. Infusion phase: Smoke and smoke substances will be applied during phase II together with the endotoxin infusion. Parameters and assays as under I.

III. Postinfusion phase: Smoke and smoke substances will be applied in phase III after terminating the endotoxin infusion. 1) Histological sections and

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

histochemical sections in the second kidney will be compared to the kidney after termination of the infusion (control). This experiment will be done in order to evaluate inhibition or activation of the reactive thrombolytic recovery phase. Additional assays as under 1.

Assays: 1) Histological section in thekidney: 100 glomeruli will be counted in each section and the number of glomerula involved with fibrin deposition expressed in %. 2) Fibrin layer according to Todd (J. Bacteriol.,78:281). I am enclosing a detailed procedure of the method. See also, Beller et al.,1961 and 1967. The coagulation assays are described in Beller and Porges,1967. 3) Activator assay on tissue extracts measured on fibrin plates (Astrup and Muellertz, Lit. in Albrechtsen,O.K.: Fibrinolysis in the organism . Acta Physiol. Scand. Suppl. 165, 1959).

1003547027

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Technique: (Mitchell, Weiss and Beller, 1967).

Preparation of fibrin film:

Bovine fibrinogen highly contaminated with plasminogen (Irving Weeks, American Hoechst) is dissolved in Michaelis Veronal buffer (pH = 7.35) to a concentration of 300 mg to 500 mg per 10 cc. 20 units of Parke Davis Topical thrombin in 1 cc of buffer is added to a test tube containing 10 ml of fibrinogen solution. The tube is inverted gently to mix the solutions and is then poured uniformly onto an 8 cm x 14 cm piece of wettable cellophane (dialysis paper) which has been saturated with the Veronal buffer and placed on a perfectly level surface. The solution is permitted to clot at room temperature and is then placed in a refrigerated (4°C) wet chamber for at least one half hour. 2 x 3 cm pieces of the film may then be cut from the 8 x 14 cm piece using a siliconized surgical scissor. These sections are inverted on clean microscope slides using care so that no bubbles form under the film. The cellophane is then pealed off leaving a 2 mm fibrin film on the slide. These films are stable, when refrigerated, for three days. Activator inhibitors may be incorporated into the films prior to the addition of thrombin. If the films are to be heated to destroy the plasminogen (86°C/30 min.), ~~these~~ The steps should be carried out in a wet chamber after the fibrin has been placed on the slide.

Tissue activator assay:

The tissue used in this assay must be received fresh, and immediately quick frozen onto a cryostat block. In this state the tissue is stable for several days if it is sealed in parafilm to prevent drying.

The tissue sections are then cut 8 microns thick and placed on the fibrin film slides with a precooled forceps. If many films are to be run, they are placed in the refrigerated wet chamber and their incubations all commense

1003547028

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

simultaneously. The incubation is carried out in a moist 37°C incubator for varying periods of time. A control slide (0 min incubation) is prepared for comparison and to aid in interpretation of the results. Incubations of relatively inactive tissues have been carried out in this laboratory for periods of up to 24 hours. The reaction is arrested by placing the slides into a 10% formaline-saline solution.

Staining procedure:

1) rinse slides in tap water bath
2) place in acid hematoxylin 90 sec. (or until red-blue color results)
3) rinse in water
4) develop in dilute ammonia water until blue
5) rinse in water
6) 50% ethyl alcohol x 15 min.
7) 70% ethyl alcohol x 15 min.
8) 95% ethyl alcohol x 30 min.
9) 100% ethyl alcohol x 45 min.
10) eosin Y x 20 sec.
11) wash in 100% ethyl alcohol three times
12) 50% ethyl alcohol - 50% xylol x 20 min.
13) 100% xylol x 20 min.
14) Trim excess fibrin film from the slides with sharp knife and cover with synthetic mounting medium and a cover slip. The entire area under the cover slip must be filled with mounting medium to prevent drying of the film. The slides are now stable, and may be stored for further reference

Comment:

The slides are best observed with a low power stereoscopic microscope. Lysed zones will first appear as depressed areas under the tissue section.

1003542029

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Slides incubated for longer periods will have holes through the entire fibrin film at areas under the tissue that correspond to the earlier depressions. Lysis on heated films or those containing activator inhibitors (e.g. 4×10^{-4} M EACA) is indicative of proteolytic activity in the tissue not due to plasminogen activator.

Semi-quantitative comparisons of activator activity in different tissues may be made with reference to the time of incubation necessary to produce a certain observed amount of lysis. However, these comparisons must be made only between slides which are prepared with the same batch of fibrinogen on one film and with simultaneous incubations.

It is preferable to use one batch of fibrinogen in which the plaminogen contamination is known ~~(for instance Behring Werke, Marburg Germany; American Hoechst, New York).~~ The method is not a histochemical assay in the true sense of the definition since the plasmin spreads. The localization is therefore dependent on a proper incubation time. Excessive incubation of the slides results in autolysis of the entire tissue section.

1003547030

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#628 FINLEY

1003547031

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 17, 1967

MEMORANDUM

TO: The committee comprising Dr. Sommers, Chm., Dr. Loosli, Dr. Reimann and Dr. Bing.

FROM: Robert C. Hockett

SUBJECT: New grant application from Theodore N. Finley, M.D. and Aaron J. Ladman, Ph.D. - No. 628.

We enclose herewith a new grant application from Drs. Theodore N. Finley and Aaron J. Ladman of the University of New Mexico School of Medicine, Albuquerque, New Mexico.

The application is supported by reprints of four recent papers on related subjects by the applicants:

1. Comparison of the Composition and Surface Activity of "Alveolar" and Whole Lung Lipids in the Dog. T.E. Morgan, T.N. Finley and H. Fralkow. Biochem. Biophys. Acta 106, 403 (1965).

2. Alterations in Pulmonary Surface Active Lipids during Exposure to Increased Oxygen Tension. T.E. Morgan, T.N. Finley, G.L. Huber and H. Fralkow. J. Clin. Invest. 44, 1737 (1965).

3. Bronchopulmonary Lavage in Normal Subjects and Patients with Obstructive Lung Disease. T.N. Finley, E.W. Swenson, W.S. Curran, G.L. Huber and A.J. Ladman. Ann. Internal Med. 66, 651 (1967).

4. The Fine Structure of the Ductuli Efferents. A.J. Ladman. The Anatomical Record 157, 559 (1967).

General evaluation of the scientific merits of the proposal is left to the committee. Staff questions are:

1. To what extent would this study duplicate or supplement others now in progress under CTR or AMA sponsorship?

2. If cigarette smoke inhalation by dogs is an essential feature of the project, do we have suitable techniques for monitored and controlled administration of whole, fresh, "normal" smoke? Are the applications willing to accept the best suggestions and guidance that the CTR staff can provide in this area from accumulated experience?

1003547032

3. In their experimental design will they distinguish between chronic differences between smokers and non-smokers and short-term effects of recent smoke exposure?

R. C. H.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PHYSIOLOGY OF THE RESPIRATORY SYSTEM

NO. 628

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Sommers, Chm.
Dr. Loosli
Dr. Reimann
Dr. Bing

Approved

Application For Research Grant

Date: August 10, 1967

1. Name of Investigator(s): (include Title and Degrees)
Theodore N. Finley, M.D., Associate Professor of Medicine
Aaron J. Ladman, Ph.D., Professor and Chairman, Department of Anatomy

2. Institution & Address:
we enc.
The University of New Mexico School of Medicine
915 Stanford Avenue N.E.
Albuquerque, New Mexico 87106

3. Short Title of Project:
Effect of cigarette smoking on lipids and morphology of alveolar lining material and macrophages.

4. Proposed Starting Date:
January 1, 1968

5. Anticipated Duration of this Specific Study:
3 years to December 31, 1970

6. Brief Descripton of Objectives or Specific Aims:
Four studies will be undertaken:

(a) Determination of the effect of cigarette smoking on the lipids and morphology of alveolar lining material and macrophages recovered by saline lavage of smoking and non-smoking humans.

(b) Determination of acute and chronic effects of smoking on lipids and morphology of dog alveolar lining material and macrophages in situ as well as on fractions recovered by saline lavage.

(c) Determination of the effect of cigarette smoking on the incorporation of radioactive lipids into alveolar lining material and macrophages of the dog.

(d) Determination of the origin of the alveolar macrophage by labelling circulating white cells with radioactive lipids and amino acids and monitoring the transport of these labelled cells into the alveoli of the lung by radioautographic microscopy.

1003547033

7. Give a Brief Statement of your Working Hypothesis:
(See attached sheet)

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See Attached Pages

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

See Attached Pages

10. Additional Requirements:

See Attached Pages

11. Biographical sketches of all principal and professional personnel (append)

See Attached Pages

12. List of publications: (Five most recent as pertinent) (append)

See Attached Pages

10035472034

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

7. WORKING HYPOTHESIS

Preliminary experiments have shown morphologic differences in the alveolar macrophages recovered by saline lavage of smokers when compared to non-smokers. The alveolar macrophages of smokers contain an increased number of inclusion bodies of various types. In addition, the fatty acid distribution patterns of the phospholipids extracted from both the cellular and non-cellular fractions of the lavage differ in smokers and non-smokers. There appear to be more unsaturated long chain fatty acids in the phospholipids of smokers. More studies are necessary to confirm these observations.

The incorporation of labelled lipids and their precursors in alveolar macrophages of control dogs and dogs exposed to cigarette smoke will be studied to determine altered lipid pathways. We feel the above studies are necessary before more detailed studies of alterations in lung morphology and lipid metabolism in patients with various lung diseases are undertaken.

In addition, studies to determine the origin of the alveolar macrophage will be attempted because smoking may alter not only the quality but also the quantity of these cells.

8. EXPERIMENTAL DESIGN AND PROCEDURES:

Surface activity of the acellular alveolar lining material will be measured on a modified Wilhelmy balance.

Lipids will be extracted from the cellular and acellular alveolar lining material by several Folch washes using $CHCl_3$:MeOH:H_2O. The various classes of lipids (neutral and phospholipids) will be separated by thin layer chromatography using silica gel H and $CHCl_3$:MeOH:acetic acid for phospholipids and petroleum ether:diethyl ether:acetic acid for the neutral lipids. The lipids will be localized on the thin layer plates with 2,7 dichlorofluorescein and removed by scraping. Fatty acid methylesters will be formed by refluxing in MeOH and H_2SO_4 and will then be separated by gas liquid chromatography at 210° using a butanediol succinate column.

The lavage sediment containing alveolar lining material and cells will be centrifuged at 10,000 x G at 0° C for 20 minutes, fixed in cold 2.5% glutaraldehyde buffered with 0.15 M Na cacodylate to pH 7.2 for two hours, washed several times in buffer, fixed in cold phosphate buffered 1% OsO_4 for two hours, rapidly dehydrated in ethanol and then embedded in Epon 812. Sections for light and electron microscopy will be made from the epon blocks.

In attempts to isolate relatively pure monocytes from circulating blood, buffy coats from dog blood obtained by exsanguination will be processed by an electronic separator so that monocytes can be separated. They will be labelled with radioactive substances and injected into dogs. Their course into the alveolar spaces will be followed by appropriate radioautographic microscopy.

The experimental design will employ the procedures outlined in the first three paragraphs above, on humans who are smokers; non-smokers will serve as controls. (The appended reprints contain further information about our methods and observations and are included as an aide for the reviewer)

100354035

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

9. PHYSICAL FACILITIES AVAILABLE

The Department of Anatomy has approximately 4,500 square feet of research space which is available for use in support of the outlined program. This includes three electron microscopes, four ultramicrotomes, two fully equipped photographic darkrooms with the supporting equipment to do excellent photomicrography. Suitable supporting technical and secretarial personnel are currently employed to assist in the implementation of the program.

Members of the Department of Medicine have laboratory areas within the medical complex. Dr. Finley's laboratory is composed of 1000 square feet and has the equipment and facilities to accomplish the biochemical procedures as outlined.

Sufficient animal quarters are present to provide adequate space to house and maintain the needed supplies of dogs.

Rooms are available at the Bernalillo-County-Indian Hospital and Albuquerque Veteran's Administration Hospital, the University's teaching hospitals, to perform the lung lavages on human subjects. X-ray and fluoroscopic equipment are at hand to monitor the positioning of the intraalveolar catheter.

10. ADDITIONAL REQUIREMENTS

We are attempting to survey a sufficiently large number of humans so that a base-line for the non-smoker and the smoker can be established. So far, over fifty subjects have undergone saline lavage. In the past, we have paid $25.00 to each individual who has volunteered for the lung lavage procedure. In the event an untoward reaction should occur, the hospital will undertake the maintenance and care of the patient. Additional funds to cover such added costs should be budgeted so that effective patient-care can be provided.

10035470 36

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Theodore N. Finley, M. D.

DATE OF BIRTH: R R

PLACE OF BIRTH: Seattle, Washington

EDUCATION: University of Washington, 1944-1950
Degree: B. S. (Chemistry)

Medical: Johns Hopkins Medical School, 1950-1954
Degree: M. D.

Internship: San Francisco County Hospital, 1954-1955

Residency: University of California Medical Center, 1955-1956
Assistant Resident in Medicine

San Francisco County Hospital, 1956-1957
Assistant Resident in Medicine

POSITIONS: Research Fellow, 1957-1958
University of Buffalo
Department of Physiology

Clinical Instructor in Medicine, 1958-1961
Cardiovascular Research Institute
University of California Medical Center

Associate Staff Member, 1960-1961
Cardiovascular Research Institute
University of California Medical Center

American Trudeau Society Research Fellow, 1958-1961

Assistant Professor of Anesthesiology, Physiology and Biophysics; Director of Anesthesiology Research University of Washington School of Medicine 1961; Associate Professor, July 1964

Associate Professor of Medicine, University of New Mexico School of Medicine, September 1964 to present.

SOCIETIES: American Physiological Society
Western Society for Clinical Research
American Society of Clinical Investigation
American Federation for Clinical Research

10035470327

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIBLIOGRAPHY - T. N. Finley, M. D.

1. Morgan, Thomas E., T. N. Finley, Gary L. Huber, and Helen Fialkow. Alterations in Pulmonary Surface Active Lipids during Exposure to Increased Oxygen Tension. J. Clin. Invest. 44:1737, 1965.

2. Morgan, Thomas E., Theodore N. Finley and Helen Fialkow. Comparison of the Composition and Surface Activity of "Alveolar" and Whole Lung Lipids in the dog. Biochim. Biophys. Acta, 106:403-413, 1965.

3. Huber, G. L., Edmunds, L. H. and Finley, T. N. Acute effects of saline lavage on pulmonary mechanics and morphology. J. Clin. Invest. (In Press)

4. Finley, T. N., Swenson, E. W., Curran, W. S., Huber, G. L. and Ladman, A. J. Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. Ann. Int. Med. 66:651, 1967.

5. Finley, T. N., Ladman, A. J., Brewer, L., and McKay, M. B. A morphological and lipid analysis of the alveolar lining material in the dog. J. Lipid Res. (In Press)

10035472038

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Aaron J. Ladman, Ph. D.

DATE OF BIRTH: R R

PLACE OF BIRTH: Jamaica, New York, U. S. A.

EDUCATION:
New York University, New York, N. Y. A.B., 1947
Indiana University, Bloomington, Ind. Ph. D., 1952

HONORS:
American Cancer Soc., Postdoctoral Research Fellow 1952-1955;
Special Research Fellow of U.S.P.H.S. 1955-1957;
Career Development Award 1962-64;
Visiting Summer Investigator, Jackson Laboratory, Bar Harbor, Maine 1949-1951, 1953, 1954, 1956, 1957, 1959;
Fellow of American Association for the Advancement of Science, 1966.

MAJOR RESEARCH INTEREST: Functional Cytology and Cytochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE:
Research Assistant, Jackson Laboratory, Bar Harbor Maine, 1947-1948;
Teaching Fellow in Anatomy, University of Cincinnati, Department of Anatomy, Cincinnati, Ohio, 1949-1949;
Teaching Fellow in Anatomy, Indiana University Department of Anatomy, Bloomington, Indiana, 1949-1952;
Research Fellow in Anatomy, Harvard Medical School, Department of Anatomy, Boston, Mass., 1952-1955;
Associate-in-Anatomy, Harvard Medical School, Department of Anatomy, Boston, Mass., 1955-1961;
Associate Professor of Anatomy, University of Tennessee Medical Units, Memphis, Tennessee, 1961-1964;
Professor of Anatomy, Chairman, Department of Anatomy, The University of New Mexico School of Medicine, 1964 to present.

10035542039

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIBLIOGRAPHY - A. J. LADMAN, Ph.D.

1. Ladman, A.J. and Finley, T.N. Electron microscopic observations of pulmonary surfactant and the cells which produce it. Anat. Rec. 154:372,1966. (Abstract)

2. Finley, T.N., Swenson, E.W., Curran, W.S., Huber, G.L. and Ladman, A.J. Bronchopulmonary Lavage in Normal Subjects and Patients with Obstructive Lung Disease. Ann. Int. Med. 66:651,1967.

3. Ladman, A.J. The fine structure of the ductuli efferentes of the opossum. Anat. Rec. 157:559,1967.

4. Finley, T.N., Conley, G.R., Huber, G.L. and Ladman, A.J. Lipid Composition of human alveolar lung material and cells. Clin. Res. 14:365,1966. (Abstract)

5. Ladman, A.J., Pratt, S.A., and Finley, T.N. Exfoliative cytology of the lung alveolus: Preliminary electron microscopic observations on cells obtained in vivo from human lungs. Elec. Micr. Soc. Am. 1967. (In Press)

10035420 40

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Dr. Susan A. Pratt - electron microscopist	100.	R R
Technical		
Laboratory Technician	100	R
Secretarial assistance	25	
Sub-Total		R
B. Consumable Supplies (list by categories)		
Chemicals	500	
Glassware	500	
Reagents	500	
EM supplies	1,000	
Sub-Total		$2,500
C. Other Expenses (itemize)		
Animals, maintenance & procurement	1,000	
1/2 service contract for EM	700	
Consulting fee to Dr. Swenson	500	
Patient reimbursements	3,000	
Sub-Total		$5,200
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)		$3,405
Total		$26,105

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	2,500	4,200		3,368	25,818
Year 3	R	2,500	4,200		3,495	26,795

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [redacted] Date
Director of Project
Telephone

Signature [redacted]
Business Officer of the Institution
Telephone

1003542041

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Fine structure of tissues during transport behavior	National Institutes of Health Grant #1 R01 GM14435-01	$38,435	9-1-66- 8-31-67
ibid.	ibid. Grant #1 R01 GM14435-02	$28,676	9-1-67- 8-31-68
Respiratory Distress - Role of Lung & Blood Lipids	National Institutes of Health Grant #5 R01 HE09491-04	$28,500	9-1-67- 8-31-68

Pending

10035470A2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#629 ERICKSON

1003547043

Source: https://www.industrydocuments.ucsf.edu/docs/ptch0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. August 18, 1967

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Little and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: New grant application from Carlton K. Erickson, Ph.D. - No. 629.

We enclose herewith a new grant application from Dr. Carlton K. Erickson of the School of Pharmacy, The University of Kansas, Lawrence, Kansas. It is supported by reprints of two studies as follows:

1. C.K. Erickson and R.K.Chalmers. Hippocampal Theta Rhythm Involvement in Cholinergic-Induced Blockade of Discriminated Avoidance Respondingin Rats. Arch. Int. Pharmacodyn, 163, 70, (1966).

2. Carlton K. Erickson. Facilitated Responding in a Discriminated Lever Press Avoidance Situation. Psychom. Sci., 8, 37, (1967).

This is the third new application for the support of studies concerning the effects of nicotine on learning, on the agenda for our September meeting. (See application Nos. 622 and 623). We are already supporting several projects in the same general area.

It is interesting to note the number of different experimental approaches which have been designed for the study of the influence of nicotine on learning. This proposal describes a well controlled research plan which looks good.

R.C.H.

1003547044

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

I. CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY
II. EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC

NO. 629

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Little
Dr. Jacobson

Application For Research Grant

Approved

Date: August 7, 1967

1. Name of Investigator(s): (include Title and Degrees)

Carlton K. Erickson, Assistant Professor of Pharmacology and Toxicology, B.S., M.S., Ph.D.

2. Institution & Address:

School of Pharmacy
The University of Kansas
Lawrence, Kansas 66044

3. Short Title of Project:

Mechanism of Learning Facilitation by Nicotine

4. Proposed Starting Date:

November 1, 1967

5. Anticipated Duration of this Specific Study:

12 months

6. Brief Descripton of Objectives or Specific Aims:

For obvious social and medical reasons, nicotine is one of the common drugs which has been studied for its effect on learning and performance. In humans who smoke regularly and inhale a significant amount of nicotine, obvious questions arise concerning the effects of prolonged nicotine absorption on the subject's alertness and learning ability. Obviously, nicotine would be considered socially unacceptable if it seriously impaired alertness and learning. Recent research, however, has demonstrated that while large doses of nicotine depress learning, small doses can enhance performance in rats and mice (1,3). Unfortunately, there has not been a concerted effort to study the true mechanism of nicotine's observed facilitation of learning. McGaugh and Petrinovich (9) have cautioned against assuming that changes in performance reflect actual changes in efficiency of the neural processes involved in learning (i.e., the so-called formation of the memory trace). It is of special practical importance with nicotine to differentiate a transient change in motivation or alertness from the relatively permanent alteration of neural processes involved in learning.

Experience has shown that in order to convincingly demonstrate that nicotine (or any centrally-active drug) is actually affecting neural learning mechanisms, 3 important criteria must be fulfilled:

1. The phenomenon of "drug dissociation" must be ruled out. McGaugh and Petrinovich (9) have stated that "subsequent studies of nicotine (on learning) should take into account the possibility that dissociation may occur with this compound". Drug dissociation refers to the situation in which habits learned by subjects in a drugged state do not transfer to the normal state, but can be evoked again whenever the subject is drugged. In other words, nicotine may act as a secondary stimulus during learning.

(to page 1a)

7. Give a Brief Statement of your Working Hypothesis:

Nicotine enhances central nervous processes involved in learning and does not demonstrate deleterious effects on behavioral performance in doses comparable to those involved in smoking.

1003542045

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Objectives and Specific Aims (continued):

of a response or other information; when the drug is not present, the learned material may not be recalled.

2. Peripheral actions of the drug must be ruled out. It is well known that changes in peripheral functioning such as autonomic and ganglionic transmission changes, sight and hearing changes, etc. can influence learning. These must be shown to be inoperative in any suspected drug effects on the learning process.

3. The contribution of central "side effects" must be determined. In order that the effect of a centrally-active drug be pinpointed to its action on central learning processes, other central effects such as changes in motivation (hunger, thirst) and excitability level (anxiety, confusion, hyperesthesia) must be ruled out. Contribution of these effects to observed performance would not be considered part of true learning alteration since they are temporary and do not directly influence permanent memory.

This project, then, is designed to fulfill the preceding criteria for nicotine by a series of appropriate pharmacologic and neurophysiologic experiments. Since the hippocampus, a limbic system structure, has been implicated by many workers as a major brain area involved in learning, this as well as other suspected structures (cortex, reticular activating system) will be concentrated upon. It is interesting that low doses of nicotine which enhance learned performance in animals also produce a characteristic theta rhythm (4-7 cps slow wave activity) in the hippocampal EEG (8). Conversely, high doses of nicotine which block learned behavior can elicit seizure activity in the hippocampus. Although these observations have never been correlated in the same animal, they provide an interesting theoretical basis for studying the relations between nicotine, learning, and the hippocampus and other related brain structures.

A unique technique to be used in this study concerns the "chronic" administration of small doses of nicotine during a relatively lengthy avoidance training session. Because of the short duration of action of nicotine, rats will be fitted with chronically-indwelling intravenous catheters for repeated nicotine administration. It is felt that this will also closely parallel the effects of a chain smoker performing a relatively complex mental or physical task.

1003547046

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

Animals - Rats will be used throughout the study, in order to satisfy current housing requirements and to allow data accumulation on a large number of inexpensive subjects.

Training procedure - Since most of the published behavioral studies with rats and nicotine have utilized either the shuttlebox or pole-climb avoidance response, it seems best to expand upon these results with a similar behavioral situation. This worker is most familiar with the discriminated lever-press avoidance response and its interpretations, so this training situation will be used. The procedure is described in detail in an accompanying reprint (Psychonom. Sci., 1967). The procedure is especially advantageous for learning studies because acquisition of the response is relatively slow, thereby allowing accurate observation of changes in response rate due to drug treatment.

Drug administration method - Since the purpose of this project is to study the effects of nicotine on learning rather than on previously-learned behavior, nicotine and saline (control) will be administered during training sessions only. That is, doses will be administered up to the time when a preset criterion is reached (either 85% avoidance average for 3 consecutive sessions, or 15 sessions, whichever is reached first). Although doses, onsets, and durations will have to be determined for our particular test conditions and animals, the literature does provide

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location) (to page 2a)

The applicant has at his disposal for this project the following facilities and equipment:

1-Staff office (air conditioned)
1-Laboratory-office combination containing desks for 5 students and animal surgery area (air conditioned) (to page 2c)

10. Additional Requirements:

For completion of programming equipment in available test chambers:

2-Grason Stadler shocker-scramblers, Model E1064GS
1-Heavy-duty power supply, 28VDC (Grason Stadler), Model E783DA
2-Lehigh Valley basic interval timer, Model 1309
1-Lehigh Valley session timer, Model 1350C
1-Lehigh Valley digital counters, Model 1425-10

11. Biographical sketches of all principal and professional personnel (append)

See page 2d.

12. List of publications: (Five most recent as pertinent) (append)

See page 2e.

1003547047

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Experimental Design and Procedures (continued):

information which can be used to design the dose schedule. Domino (3) has shown that nicotine in relatively small doses reaches its peak after 5 minutes and has a duration of approximately 30 minutes. Since the avoidance training method to be used requires 4-hour sessions every other day, it is obvious that nicotine will have to be given more than once in order to provide prolonged drug action for an entire session. Thus animals will be fitted with chronic-indwelling catheters via the jugular vein according to a method described by Weeks and Davis (11) and modified by us (5). The cannula will be attached to a swivel fluid-lock assembly mounted in the top of the test chamber which will allow the animal to move freely without twisting the cannula. Injections will then be made at desired intervals without physically interrupting the animal's training session.

Doses to be used - According to Johnston (6), 1/50 gr. of intravenous nicotine produces, in a habitual smoker, the same psychic effects as smoking one cigarette. Assuming this effect occurs in a 70 kg. man, the "cigarette equivalent" of nicotine received is calculated to be 18.6 ug/kg. of body weight. Since our experimental design will utilize intravenous nicotine administration, the nicotine "cigarette equivalent", as well as other similar small doses, will be thoroughly investigated. Along these lines, it is interesting that the doses used by Domino (3) to facilitate avoidance behavior (40-80 ug/kg. subcutaneously) and by Brown (2) to evoke hippocampal responses (30-50 ug/kg. subcutaneously) are similar to our calculated cigarette equivalent. It further seems very possible that our 18.6 ug/kg. dose given intravenously may produce the same effects as the Domino and Brown subcutaneous doses.

Experimental plan - The 3 criteria discussed under Objectives will be investigated using nicotine as follows:

1. Drug dissociation - In order to determine the presence or absence of nicotine dissociation of learning, groups of rats will be trained in the conditioned avoidance situation under the effects of nicotine and saline. Rate of response acquisition to the preset criterion will be recorded to determine enhancement of response rate with nicotine. Later sessions will consist of performance measurement of both groups treated with saline. Drug dissociation will be ruled out if the nicotine-trained rats continue to perform at the pre-saline level.

2. Central action of nicotine - The usual method of determining whether a drug is acting centrally is to demonstrate a behavioral action with the drug, followed by loss of the action with a quaternary derivative which will not pass the blood-brain barrier. In this regard, Domino (4) has reported that congeners such as nicotine methiodide are relatively ineffective in contrast to nicotine in blocking conditioned avoidance behavior in rats. Nicotine methiodide will be used to confirm these results in our conditions, and to control for central specificity in other parts of the study.

Another method to test for specificity of drug effects in the brain is simply to place the drug into various structures of interest by means of a cannula. This provides a means of determining significant differences in drug effect from parenteral administration. Using apparatus similar to that described above for intravenous injections, rats with chronically-indwelling cannulas in the brain will be tested with small doses of nicotine injected into the cortex, hippocampus, and reticular formation. Intracerebral quantities of nicotine will approximate 1×10^{-9} moles, and solution volumes will be not more than 2 uL/injection. A modification of the nylon-stainless steel assemblies of Valenstein et al. (10) will be used for cannula construction, and nicotine will be administered intracerebrally at times comparable to parenteral administration.

1003547048

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Confirmation of cannula placements will be performed histologically. Frozen sections of 50 micron thickness will be cut, placed on slides, and stained with cresylechtviolett and methylene blue according to the method of Kluver and Barrera (7).

3. Direct action on neural learning processes - The procedure of administering drugs after training rather than before training has been adopted by a number of investigators as a means of assessing memory storage effects by ruling out motivational changes and excitability factors. A current popular theory of learning and memory states that stimuli entering the CNS initiate reverberating neural pathways which must continue for a fixed time to form a memory trace. The longer the pathway reverberates and the stronger the incoming stimulus, the more deeply the memory trace is implanted. Thus any overriding stimulus (e.g., electroshock or certain drugs) which disrupts the reverberating neural pathway (assuming the pathway is electrical-chemical in nature) will inhibit learning and trace formation. Post-trial electroshock and various drugs such as barbiturates and tranquilizers have indeed been shown to affect the learning process in this manner (9). On the other hand, central stimulants such as emphetamine and strychnine given after training trials in animals have been shown to enhance the learning process (9). This technique is especially suitable for drugs which are maximally effective within minutes and which are metabolized prior to retention tests, as is nicotine. Thus, enhancement of conditioned avoidance learning when nicotine is given after each session would strongly indicate facilitation of memory trace formation by this drug.

References

1. Bovet, D., Bovet-Nitti, F., and Oliverio, A., "Effects of nicotine on avoidance conditioning of inbred strains of mice", Psychopharm. 10: 1-5 (1966).

2. Brown, B.B., "Relationship between evoked response changes and behavior following small doses of nicotine", Ann. N.Y. Acad. Sci. 142:190-200 (1967).

3. Domino, E.F., "Some behavioral actions of nicotine", in Tobacco Alkaloids and Related Compounds, ed. Von Euler, The MacMillan Company, New York (1965), pp. 145-62.

4. Domino, E.F., "Some comparative pharmacological actions of (-)-nicotine, its optical isomer, and related compounds", in Tobacco Alkaloids and Related Compounds, ed. Von Euler, The MacMillan Company, New York (1965), pp. 303-13.

5. Erickson, C.K., unpublished data on chronic intravenous alcohol infusion in rats.

6. Johnston, L.M., "Tobacco smoking and nicotine", Lancet 243:742 (1942).

7. Kluver, H. and Barrera, E.A., "A method for the combined study of cells and fibers in the nervous system", J. Neuropathol. exp. Neurol. 12: 400-3 (1953).

8. Longo, V.G., Giunta, F., and Scotti de Carolis, A., "Effects of nicotine on the electroencephalogram of the rabbit", N.Y. Acad. Sci. 142:159-69 (1967).

1003547049

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

9. McGaugh, J.L. and Petrinovich, L.F., "Effects of drugs on learning and memory", Intern. Rev. Neurobiol. 8:139-96 (1965).

10. Valenstein, E.S., Hodos, W., and Stein, L., "A simplified electrode-assembly for implanting chronic electrodes in the brains of small animals", Am. J. Psychol. 74:125-8 (1961).

11. Weeks, J.R. and Davis, J.D., "Chronic intravenous cannulas for rats", J. Appl. Physiol. 19:540-1 (1964).

Physical Facilities Available (continued):

1-Behavioral laboratory (air conditioned) containing 9 rat test chambers and relay programming equipment (3 test chambers will be available for this project; some programming equipment is required to complete the chambers - see Additional Requirements)
1-Histological laboratory (for confirmation of cannula placement work), with microtome equipped for carbon dioxide and paraffin sectioning

10035420S0

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Recent Publications:

Chalmers, R.K. and Erickson, C.K., "Central cholinergic blockade of the conditioned avoidance response in rats", Psychopharmacologia 6:31-41 (1964).

*Erickson, C.K. and Chalmers, R.K., "Hippocampal theta rhythm involvement in cholinergic-induced blockade of discriminated avoidance responding in rats", Arch. int. Pharmacodyn. 163:70-8 (1966).

*Erickson, C.K., "Facilitated responding in a discriminated lever press avoidance situation", Psychonom. Sci. 8:37-8 (1967).

Erickson, C.K., "A reliable lever-press avoidance training method in the rat", Fed. Proc. 26:738 (1967).

Patel, J. and Erickson, C.K., "Enhanced avoidance acquisition by low-intensity hippocampal stimuli", Pharmacologist 9:200 (1967).

* Reprint accompanies proposal

1003547051

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Carlton K. Erickson, Prin. Invest.		
9 months academic year	20%	Not requested
3 months summer	100%	R
Fringe benefits		
Technical		
Jitendra B. Patel, Graduate Student		
12 months calendar year	50%	$ R
Sub-Total		$ R
B. Consumable Supplies (list by categories)		
Animals and feed		$ 400
Cannula materials		100
Drugs and histological chemicals		100
Recorder paper, notebooks		50
Sub-Total		$ 650
C. Other Expenses (itemize)		($ 7,075)
None		
Sub-Total		
D. Permanent Equipment (itemize)		
2-Grason Stadler Shockers with Grid Scrambler, Model E1064GS @ $ 285.00		$ 570
1-Grason Stadler Heavy-duty Power Supply, 28VDC, Model E783DA		175
2-Lehigh Valley Basic Interval Timers, Model 1309 @ $ 95.00		190
(to page 3a) ... Total-		$ 1,214
E. Overhead (16% of A+B+C)		$ 1,061
Total		$ 9,350

Estimated Future Requirements: NONE

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Carlton K. Erickson
Director of Project
913 UN4-4004
Telephone

Signature Raymond Nichols
Business Officer of the Institution
VICE CHANCELLOR FOR FINANCE
UN-3136
Telephone

10035472052

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

D. Permanent Equipment (continued)

1-Lehigh Valley Session Timer, Model 1350C	$ 80
1-Lehigh Valley Digital Counters, Model 1425-10	$199

10035420S3

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
"Addictive Ability of Self-injected Intravenous Alcohol in Rats"	Licensed Beverage Industries	$ 7,965	11/1/66 - 10/31/67
"Behavioral Hyperexcitability Induced by Alcohol Withdrawal in Rats"	University of Kansas Research Committee	$ 3,000	7/1/67 - 6/30/68

Pending

"Cholinergic Implications of Ethanol Intoxication"	National Institute of Mental Health	$ 11,925 ($ 35,700)	1/1/68 - 12/31/68 (1/1/68 - 12/31/70)
"Enhancement of Learning by Limbic System Stimulation"	National Institute of Mental Health	$ 25,118 ($ 66,586)	1/1/68 - 12/31/68 (1/1/68 - 12/31/70)

100354705A

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#630 PARSHLEY

1003547055

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

August 18, 1967

Approved

MEMORANDUM

TO: The committee comprising Dr. Loosli, Chm., Dr. Sommers, Dr. Reimann and Dr. Little.

FROM: Robert C. Hockett

SUBJECT: New grant application from Mary Stearns Parshley, Ph.D. - No. 630.

We enclose herewith a new grant application, supported by two reprints, from Dr. Mary Stearns Parshley of Columbia University College of Physicians and Surgeons, New York, New York.

A well documented application for the support of a broad study of the effects of the constituents of tobacco smoke on normal and malignant human respiratory epithelium in vitro.

R. C. H.

Reprints:

1. Mary Stearns Parshley and Ines Mandl. - Inhibition of Growth of Malignant Cells in vitro by a Component of Normal Adult Connective Tissue. Nature, 208, 5012 (1965).

2. Effect of Inhibitors from Adult Connective Tissue on Growth of a Series of Human Tumors in vitro. Mary Stearns Parshley. Cancer Research, 25, 3 (1965).

10035470S6

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

NO. 630

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Doosli, Chm.
Dr. Sommers
Dr. Reimann
Dr. Little

Application For Research Grant

Date: August 10, 1967

1. Name of Investigator(s): (include Title and Degrees): Mary Stearns PARSHLEY, Ph.D.
Assistant Professor of Anatomy in Obstetrics & Gynecology

2. Institution & Address: Columbia University
College of Physicians & Surgeons

630 West 168th Street
New York, N. Y. 10032

3. Short Title of Project:

EFFECT OF CONSTITUENTS OF TOBACCO SMOKE ON NORMAL AND MALIGNANT HUMAN RESPIRATORY EPITHELIUM IN VITRO.

4. Proposed Starting Date: November 1, 1967

5. Anticipated Duration of this Specific Study: Although some meaningful results should be obtained by the end of the first year, it is expected that an additional period will be required.

6. Brief Description of Objectives or Specific Aims:

There is an increasing emphasis on tobacco smoke as a contributory factor to the development of lung cancer. Tobacco products, the polycyclic aromatic hydrocarbons in particular, have been shown to induce or enhance neoplastic transformation in laboratory animals (4,9,15). On the other hand some constituents, namely the monocarboxylic acids, have been reported to have an anti-tumor effect (12). There is little precise knowledge of the qualitative and quantitative effects on normal respiratory epithelium of the nearly 300 organic and inorganic compounds reported as constituents of tobacco smoke (4,8). These include metals, hydrocarbons, alkaloids chiefly nicotine, nitrogen products, and volatile elements. Either similar factors found in polluted air or still other factors must be responsible for the occurrence of lung cancer in non-smokers, and the higher incidence of this disease among male smokers. A great deal of study has been devoted to the biological activity of tobacco tars or condensates with emphasis on the polycyclic hydrocarbons which are present only in traces (1,2,5,6,9,15). Not only has it been shown that these hydrocarbons are not specifically carcinogenic for epithelium (6,9), but the fact that fractions of smoke condensate not containing hydrocarbons also produce neoplastic changes in lung epithelium has been demonstrated (6,9). Most animal experiments deal with the carcinogenic effect on skin of smoke condensates and their fractions (4,7,8,15). Studies in which animals actually inhaled smoke gave inconsistent results (7). The skin is quite different from the mucus-coated respiratory epithelium and apparently more sensitive to the polycyclic hydrocarbons. Although nicotine content has been considered to be detrimental to the cleansing activity of the respiratory cilia (3), very little is established as to the local biological effect of nicotine (4). Little attention has been paid to the volatile substances such as carbon monoxide, hydrogen cyanide, and nitrogen oxides, present in many times higher concentration than that considered to be "safe" in industry (8,14). It is possible that neoplastic changes in normal respiratory epithelium

(cont. on p.1a)

7. Give a Brief Statement of your Working Hypothesis:

If the development of malignancy in normal human respiratory epithelium is the result of an interference with the oxygen supply and normal respiration, a simulation

(cont. on p. 1a)

1003547057

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

6. cont.
may be caused by an interference on the part of some of these volatile constituents of tobacco and polluted air with the oxygen supply to these cells necessary for aerobic respiration. This anoxia could result in a mutation toward anaerobic glycolysis as a source of energy characteristic of bronchogenic carcinoma (14).

The technique of tissue culture makes possible the direct observation of human tissues under high magnifications. The biological effect of whole tobacco smoke as well as some of its volatile constituents on these cells can be studied microscopically, and the metabolic byproducts of the cells under different conditions can be analyzed. Cell lines provide a source of uniform test material. In addition test materials can be added in a concentration not possible in the intact animal and under conditions of prolonged contact permitting the equilibrium of poorly soluble or slowly diffusing substances between the medium and the cells. A number of materials possibly related to a neoplastic activity of either tobacco smoke or polluted air have been studied by this method. Hyperplasia and metaplasia considered preneoplastic in cultures of human foetal lung treated with polycyclic hydrocarbons and tobacco smoke condensate and its fractions was reported by Laznitski (5,6). Crocker and coworkers havecarried on similar studies with rat and hamster trachea in tissue culture (1,2,9). These workers reported a nonspecific effect of these carcinogens, and also that analogs of these hydrocarbons not known to be carcinogenic produced similar abnormalities in cultures of respiratory epithelium. Toxic effects of organic constituents of tobacco smoke and polluted air, acetaldehyde, nitrogen dioxide, and monocarboxylic acids, on lines of human and animal cells in tissue culture were observed by Pace and his coworkers. (10,11,12). Rounds also observed that nitrogen dioxide introduced as sodium nitrite into the medium of cultures of trypsinized and suspended cells from the lungs of mice and rats caused nuclear and cytoplasmic changes which accompanied a reversible inhibition of respiratory activity (13).

See continuation sheet 1b for Pertinent Literature Ref, cited above.

It is proposed to study the effect of some of the gaseous constituents of tobacco smoke and polluted air on established lines of normal and malignant human respiratory epithelium from the pharynx, larynx, and lung and to compare their characteristics as evidenced in tissue culture under these experimental conditions. Effect on rate of proliferation, cell movement and contact relationship, nuclear and cytoplasmic structure and chromosome content will be correlated with differences in metabolic activity and related to neoplastic capability. A clear understanding of the morphological and metabolic differences between normal and malignant human respiratory epithelium should contribute to an understanding of the neoplastic process itself..

7.,cont.
of these conditions in tissue cultures of these cells by a manipulation of the gaseous atmosphere of the culture should demonstrate this. Similar experimentation with the gaseous phase over respiratory cancer cells in tissue culture might lead to changes in the morphologic and metabolic characteristics of these altered cells which could give a clue to the causes of carcinogenesis. These studies have a direct bearing on neoplastic development and the biological and biochemical mechanisms responsible.

1003547058

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

6. cont. Pertinent Literature References

1. Crocker, T. T., Nielson, B. I., and Laznitski, I. Carcinogenic hydrocarbons. Arch. of Environ. Health, 10:2:240, 1965.

2. ----------, and Nielson, B. I. Effects of carcinogenic and non-carcinogenic hydrocarbons on respiratory epithelium in orhan culture. Tissue Culture Assn. Proc., Miami, June, 1965, p. 56.

3. Dawson, F. W. Effects of nicotine and influenza on human ciliary activity in vitro. Fed. Am. Soc. Exp. Biol. and Med. Proc., 24: part I, #260, 1965.

4. Kensler, C. J. The pharmacology of tobacco smoke effects of chronic exposure. in "Tobacco and Health", ed. by G. James and T. Rosenthal, Chap. 1, Charles Thomas, Springfield, Ill. 1962, p. 5.

5. Laznitski, I. Effect of 3,4-Benzpyrene on human foetal lung grown in vitro. Brit. J. Cancer, 10:510, 1956.

6. ---------- Observations on the effects of condensates from cigarette smoke on human foetal lung in vitro. Brit. J. Cancer, 12:547, 1958.

7. Leuchtenberger, C., and Leuchtenberger, R. A correlated histological, cytological, and cytochemical study of the major bronchi from mice exposed to cigarette smoke. in "Tobacco and Health", ed. by G. James and T. Rosenthal, Chap. 8, Charles Thomas, Springfield, Ill. 1962, p. 81.

8. Lindsey, A. J. Some observations upon the chemistry of tobacco smoke. in "Tobacco and Health", ed. by G. James and T. Rosenthal, Chap. 2, Charles Thomas, Springfield, Ill., 1962, p. 21.

9. Nielson, B. I., and Crocker, T. T. Epithelial and mesenchymal interactions in hamster respiratory mucosa exposed to polycyclic hydrocarbons in organ culture. Tissue Culture Assn. Proc., Philadelphia, June, 1967, p. 15.

10. Pace, D. M., and Elliott, A. Studies on the effect of acetaldehyde on tissue cells cultivated in vitro. Cancer Res., 20:868, 1960.

11. ----------, Thompson, J. R., Aftonomos, B., and Holck, G. O. Effects of NO_2 and salts of NO_2 on established cell lines. Canad. J. Biochem. and Phys., 39:1247, 1961.

12. ----------, Aftonomos, B., Elliott, A., and Sommer, S. Observations on some effects of the sodium salts of certain monocarboxylic acids on established cell lines. Canad. J. Biochem., 45:81, 1967.

13. Rounds, D. E., and Bils, R. F. Effects of air pollution on cells in culture. Arch. Environ. Health, 10:251, 1965.

14. Stevens, K. M. Lung cancer, an evolutionary approach. Austral. J. Exp. Biol. and Med. Sci., 43:421, 1965.

15. Wynder, E. L., Hoffmann, D., and Auerbach, O. The role of particulate and volatile components in tobacco carcinogenesis. Am. Assn. Cancer Res., 6:69, 1965.

1003547059

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

It is planned to maintain the following lines of normal and malignant human respiratory epithelium as monolayers of cells on glass in milk dilution bottles in mixtures of serum (human placental, horse, and calf) in synthetic media, Parker's Medium 199 or Eagle's minimal essential medium (MEM) under an atmosphere of 5% CO_2 in air.

a. Line KB, human epidermoid carcinoma of the pharynx
b. Line H.Ep.#2, human epidermoid carcinoma of the larynx, carried in our laboratory.
c. Line L-132, normal human fetal lung epithelium

We also intend to set up other lines of freshly explanted normal and malignant human epithelium from biopsy material which will be obtained through the cooperation of Dr. Sheldon C. Sommers, Professor of Pathology at Delafield Hospital. These lines will be used to compare the effect of the experimental conditions on freshly explanted tissue as opposed to the older lines.

Experiments to determine the effect of tobacco smoke and some of its volatile components will be carried out as follows. At intervals series of tubes with and without coverslips will be seeded with a known number of cells. After the cells have become confluent the medium will be renewed and the cultures will be gassed by means of an apparatus used routinely in our laboratory for adjusting the gaseous phase of the culture with mixtures of O_2, CO_2, N_2, and NO_2 for short periods. In other experi-

(cont. on p. 2a)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

Large well-equipped tissue culture laboratory with glass enclosed sterile room, incubators, refrigerators, deep-freezes, large and small centrifuges, Mettler microanalytical balances, torsion balances, Leitz Ortholux research microscope with equipment for bright light and phase contrast microscopy, Zeiss inverted research microscope with some light and phase contrast equipment, Leitz binocular microscope with Leitz Micras Time attachment for photography with lightmeter, camera back, and other equipment for microphotography and microscopy, sterilizers, stills, special glassware and equipment for Carrel flask, Maximow slide, Leighton tube, and bottle culture, equipment for histological and histochemical studies.

10. Additional Requirements:

The chemical investigation will be carried out in collaboration with Dr. Ines Mandl who has several rooms in Delafield Hospital equipped for biochemical analysis and separation, including cold room, deep freezes, refrigerators, freezer-dryers, analytical balances, homogenizers, two high speed ultracentrifuges, chromatographic and electrophoretic equipment, Spinco L2 ultracentrifuge with swinging bucket, and Elphor FF electrophorator. Dr. Mandl is Asst. Professor of Biochemistry in Obstetrics & Gynecology.

10035472060

11. Biographical sketches of all principal and professional personnel (append)

See attached curriculum vitae.

12. List of publications: (Five most recent as pertinent) (append)

See attached continuation sheet 2b.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. cont.
ments the salts, NaSCN and $NaNO_2$, will be added directly to the culture medium or to balanced salt solution in serial dilution and allowed to remain in contact with the cells for short periods of time. Cultures of freshly explanted normal human respiratory epithelium will be exposed directly to diluted fresh tobacco smoke by the same apparatus.

The effect of these substances on the cells will be determined by the following studies.

1. Growth rate studies. Effect on rate of growth will be evaluated by dye exclusion cell counts with trypan blue, and mitotic counts.

2. Morphological Studies. Studies of differences in cell morphology and in number and character of chromosomes will be made as well as histochemical preparations to demonstrate different enzyme content. Parallel cultures on coverslips in Leighton tubes will be fixed and stained with H. and E., Giemsa, Feulgen, Methyl Green and Pyronin Y to observe effect on mitosis and nucleic acid content. Enzyme content of the cells will be observed from preparations in which the cells have been reacted for sites of alkaline phosphatases, beta-glucuronidase, succinic dehydrogenase, and leucyl beta-napthylamidase activity. For chromosome studies, cultures will be treated with colchicine to halt mitosis in the metaphase, fixed, spread, stained with acetic-orcein and observed under the oil immersion objective of the microscope and photographed.

3. Biochemical Studies. The cells and culture medium will be analyzed to evaluate the differences in the metabolic by-products of the cell lines, both treated and untreated.

a. The effect on cellular respiration and glycolysis will be observed using standard Warburg techniques. In particular glucose disappearance and lactic acid production in the used culture medium will be estimated by the methods of Trevelyan and Harrison (Biochem. J., 50:299, 1952) and Hullin and Noble (Biochem. J., 55:289, 1953).

b. The cells will be fractionated in a Spinco L2 ultracentrifuge and the mitochondrial fraction extracted and studied in the Warburg apparatus by the method of Umbreit (Manometric Techniques and Tissue Metabolism, Chap. I) and the ratios of oxygen uptake to phosphorus utilization will be estimated and compared.

c. Differences in protein patterns of the metabolic products of the cells will be established by acrylamide gel electrophoresis.

d. The enzymatic profiles of both cells and utilized culture medium will be studied with special reference to proteolytic enzyme activity using chromagenic substrates and modifications of the Bratton-Marshall method (Goldberg, Pineda, and Rutenburg, Am. J. Clin. Path., 32:571, 1959; Blackwood and Mandl, Anal. Biochem., 2: 370, 1961).

10035470G1

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

12. Supporting Data:

A. Previous work done in this or related fields. The principal investigator has carried on extensive studies of the behavior of normal and malignant cells in rabbit's ear chambers and in tissue cultures. In collaboration with H. S. Simms several hundred biological substances and organic and inorganic chemicals were tested for their effect on growth of normal epithelium and connective tissue cells. More recently growth stimulating and growth inhibiting materials have been extracted from normal adult connective tissue and tested for effect on growth of human tumor biopsies, Rous sarcoma, and many lines of normal and malignant human and animal cells initiated in this laboratory and maintained in conrinuous cultivation. These materials were also tested against animal tumors in vivo, Sarcoma-180 in the mouse and Flexner-Jobling carcinoma in the rat and against heterologously transplanted human ovarian carcinoma in conditioned rats. The inhibitory material reduced the growth of malignant cells in tissue culture from 75-100%. Collateral studies showed that these factors inhibited the growthof animal tumors also. Recently by using different methods of fractionation we have separated materials with different growth inhibitory properties. We have obtained substances with specific inhibition for malignant fibroblasts in vitro and in vivo in one case, and materials primarily effective against malignant epithelium in another. The latter factors inhibited the same line of highly malignant ovarian carcinoma cells in tissue culture and also in animals when transplanted heterologously into conditioned weanling rats. Active specific growth stimulating and growth inhibiting materials have been separated from crude extracts by ion-exchange DEAE-cellulose chromatography and by gel filtration on Sephadex-G200 and Biogel-300. At the present time biochemical analysis is in progress to determine the exact chemical nature of these inhibitors.

B. Six most pertinent recent references. (Reprints of Cancer Res. & Nature papers included.)

Parshley, M. S. Tissue Culture of Adult Tissue. Chap. 17 in Vol. II of the "Transplantation of Tissues", ed. by L. Peer. Williams & Wilkins & Co. Baltimore 1959.

Simms, H. S., and Parshley, M. S. The effect of proteins and other substances on the growth of adult tissue in vitro. Chap. 7 in "Protein and Amino Acid Nutrition", ed. by A. Albanese. Academic Press. New York, 1959.

Parshley, M. S., and Mandl, I. Separation and study of a connective tissue constituent inhibitory to the growth in vitro of tumor cell strains. Am. Assoc. for Cancer Res. Proc., 4:1:50, 1963.

Parshley, M. S. Effect of inhibitors from adult connective tissue on growth of a series of human tumors in vitro. Cancer Research, 25:387, 1965.

Parshley, M. S., and Mandl, I. Inhibition of malignant cells in vitro by a component of normal adult connective tissue. Nature, 208:800, 1965.

Parshley, M. S., Mandl, I., and Manahan, J. Separation and in vitro study of tumor cell inhibitors. Tissue Culture Assn. Proc., San Francisco, June, 1966.

10035470G2

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Technical		
Research Assistant	100	R
Diener	50	
Fringe Benefits (10)		
	Sub-Total	R
B. Consumable Supplies (list by categories)		
Special glassware and equipment for tissue culture		$ 1,000
Media, chemicals, and stains		1,000
	Sub-Total	$ 2,000
C. Other Expenses (itemize)		
Travel to scientific meetings		$ 500
	Sub-Total	$ 500
D. Permanent Equipment (itemize)		
Zeiss Photomicroscope		$ 5,820
Mark Scholander Respirometer		975
E. Overhead (15% of A+B+C)		$ 1,744.50
	Total	$20,169.50

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	$ 2,000	$ 500		$ 1,835	$14,070
Year 3		$ 2,000	$ 500		$ 1,910	$14,640

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Mary L. Parshley
Director of Project
Telephone

Signature [illegible]
Business Officer of the Institution
VICE-PRESIDENT IN CHARGE OF MEDICAL AFFAIRS
Telephone

1003547063

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Study of Naturally Occurring Growth Inhibitors	National Cancer Institute	$ 28,736	to 12/31/67
	Growth Inhibition of Malignant Cells in vitro and in vivo	Damon Runyan Memorial Fund	20,900	to 2/29/68
Pending	Effect of Vinca alkaloids on Human Neoplastic Cells in tissue culture	Leukemia Society, Inc. Scholarship	75,000	Jan. 1, 1968 to Dec. 31, '72
	Biology and Chemistry of Cultured Ovarian Cancer Cells	National Cancer Institute	50,445	Jan. 1, 1968 to Dec. 31, 1970

1003547064

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Mary Stearns Parshley
Assistant Professor of Anatomy in Obstetrics and Gynecology
College of Physicians and Surgeons, Columbia University
630 West 168th Street, New York, New York 10032

R R R

Married: Thomas Fredricson Parshley, New York City, April 15, 1938
Child: Elisabeth Stearns Parshley, born June 5, 1947

Academic Background:

Smith College, A.B. with High Honors 1933 (Zoology)
University of Pennsylvania, M.A. 1935 (Anatomy, Medical Sciences)
University of Pennsylvania, Ph.D. 1938 (Anatomy, Medical Sciences)
Travelling Fellow from Smith College at the University of Penna. 1934-1938 (Wilder, Nicolson, and Fine fellowships).
University Scholar, University of Pennsylvania, 1935-1936.
Bennett Fellow, University of Pennsylvania, 1936-1937.

Professional Experience:

1. Laboratory assistant, Willard Parker Hospital, New York City, 1933-1934.
2. Fellow in Anatomy, University of Pennsylvania, 1938-1939. Finney-Howell postdoctoral research Fellow from the Johns Hopkins University, 1938-1939.
3. Research Assistant in Pathology, Columbia University, 1940-1945. Research Associate in Pathology, Columbia University, 1945-1949.
4. Research Associate in Surgery, Columbia University, 1949-1963. Assistant Professor of Anatomy in Surgery, Columbia University, 1963-1965.
5. Lecturer in Biology, Hunter College Graduate School, 1957-1963.
6. Assistant Professor of Anatomy in Obstetrics and Gynecology, Columbia University, 1965-

1003547065

Summer:
1. Smith Scholar, Marine Biological Laboratory, Woods Hole, Mass. 1932.
2. Laboratory Assistant, St. John's Hospital, Brooklyn, New York, 1934.

World War II: Office of Scientific Research and Development, 1942-1945. (Certificate, O.S.R.D., bronze plaque, Columbia University).

Special Fields of Interest: Microscopic Anatomy and Cell Physiology. The study of living cells and tissues by the Clark-Sandison rabbit's ear chamber method, and by a variety of tissue culture techniques. Wound healing, regeneration of connective tissue, skin and blood vessels, tissue transplantation, atherosclerosis, cancer research.

Societies and Committees: Phi Beta Kappa, Sigma Xi, AAAS, Am. Assn. Anat., Physiol. Soc., Phila., Tissue Culture Assn., Am. Heart Assn., Am. Assn. Study of Arteriosclerosis, Am. Soc. Cell Biol., Am. Assn. Cancer Research. Corresponding Sec., Tissue Culture Assn., 1946-1952, Member Course Committee and Registrar Tissue Culture Assn. Summer Course, 1946-1964.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS - MARY STEARNS PARSHLEY

1. Stearns, Mary L. — Growth and differentiation of connective tissue as observed microscopically in the living rabbit. Am. J. Med. Sci. 198:144-145, 1939. Proc. Phys. Soc., Phila., 14:20-21, 1938-1939.

2. Stearns, Mary L. — Growth and differentiation of connective tissue as observed microscopically in the living rabbit. Anat. Rec. 73:3, 1939 Suppl. 2, p.49 (abstract).

3. Stearns, Mary L. — Studies on the development of connective tissue in transparent chambers in the rabbit's ear. I. Am. J. Anat. 66: 133-176, 1940.

4. Stearns, Mary L. — Studies on the development of connective tissue in transparent chambers in the rabbit's ear. II. Am. J. Anat. 67: 55-97, 1940.

5. Parshley, M.S., and Simms, H.S. — Conditions favoring the growth of adult skin epithelium in vitro. Anat. Rec. 94:3:486, 1946 (abstract).

6. Simms, H. S., and Parshley, M. S. — Studies on the fat depositing mechanism. Proc. Am. Soc. Study Arteriosclerosis. Am. Heart J. 35:860-861, 1947.

7. Simms, H. S., Parshley, M. S., and Pitt, R. B. — Fat deposition in vitro caused by lipfanogens and opposed by antilipfanogen. J. Geront. 2:3:205-216, 1947.

8. Simms, H. S., Parshley, M. S., Pitt, R. B., and Fulton, J. B. — Further studies on the fat depositing mechanism in vitro. Am. Heart J. 36: 469, 1948 (abstract).

9. Fulton, J. B., Parshley, M. S., Simms, H. S. — Growth of blood vessels from adult tissue in vitro. Anat. Rec. 103-453, 1949 (abstract).

10. Parshley, M. S., and Simms, H. S. — Cultivation of adult skin epithelial cells, chicken, and human, in vitro. Am. J. Anat. 86:163-190, 1950.

11. Parshley, M. S., and Deterling, R. A., Jr., and Coleman, C. C., Jr. — Cultivation in vitro of fresh and frozen segments of the abdominal aorta of the dog. Anat. Rec. 106:64, 1950, (abstract).

1003547066

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS - continued

Mary Stearns Parshley

12. Deterling, R. A., Jr., C. C. Coleman, Jr., and Parshley, M. S. — A preliminary report on experimental studies of the frozen homologous aortic graft. N. Y. Med. 6:19-22, 1950.

13. Deterling, R. A., Jr., C. C. Coleman, Jr., and Parshley, M. S. — Experimental studies of the frozen homologous aortic graft. Surgery 29:419-440, March 1951.

14. White, J. F., and M. S. Parshley — Growth in vitro of blood vessels from the bone marrow of adult chickens. Am. J. Anat. 89:321-346, 1951.

15. Coleman, C. C., Jr., R. A. Deterling, Jr., and M. S. Parshley — Experimental studies of preserved aortic homografts. Ann. Surg. 134:868-878, 1951.

16. Hui, K.K.L., E.B.C. Keefer, R. A. Deterling, Jr., J. W. Blunt and M. S. Parshley — Early results of experimental studies of the action of high intensity electrons on aortic homografts. Surgical Forum 2:255-260, 1951. W. B. Saunders Co.

17. Pate, J. W., P. Sawyer, R. A. Deterling, Jr., J. W. Blunt and M. S. Parshley — Early results in experimental use of freeze-dried arterial grafts. Surgical Forum 3:147-152, 1952. W. B. Saunders Co., Phila.

18. Deterling, R. A., Jr., and M. S. Parshley and J. W. Blunt — A critical study of present criteria governing selection of blood vessel grafts. Surgery 33:213-232, Feb. 1953.

19. Parshley, M. S., and R. A. Deterling, Jr. — The effect of storage at low temperature on growth in vitro of adult dog aorta. Anat. Rec. 115:2:357, Feb. 1953 (abstract).

20. Parshley, M. S., R. A. Deterling, Jr., and C. C. Coleman, Jr. — Tissue culture studies of blood vessel grafts. I. The cultivation in vitro of fresh normal adult aorta (dog, cat, rabbit, goat, monkey, and human). Am. J. Anat. 93:2:221-272, Sept. 1953.

21. Parshley, M. S. — The development of tubular structures in cultures of epithelium from the cortex of adult dog kidney. Anat. Rec. 118:2:452, Feb. 1954 (abstract).

1003547067

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS - continued

Mary Stearns Parshley

22.	Coleman, C. C., Jr., R. A. Deterling, Jr., and M. S. Parshley	Some long-term observations on aortic homografts. Surgery, 37:64-79, Jan. 1955.
23.	Parshley, M. S. and R. A. Deterling, Jr.	The effect of storage at low temperatures on growth of adult dog aorta. A. J. Anat. 97:3:359-394, Nov. 1955.
24.	Parshley, M. S.	Factors affecting the character of the outgrowth of tissue culture from the bone marrow of adult human rib. Anat. Rec. 124:429, 1956 (abstract).
25.	Parshley, M. S.	The effect of serum from patients with the nephrotic syndrome on tissue cultures of human embryonic kidney. Anat. Rec. 127:439, 1957 (abstract).
26.	Parshley, M. S.	The tissue culture of adult tissues. Chap.17 in the "Transplantation of Tissues", Vol.II, Lyndon Peer, ed., Williams and Wilkins Co., Baltimore, pp.593-633, 1959.
27.	Simms, H. S., and M. S. Parshley	The effect of proteins and amino acids on the growth of adult tissue in vitro. Chap.7 in "Protein and Amino Acid Nutrition", A. Albanese, ed., Academic Press, New York, pp.143-195, 1959.
28.	Parshley, M. S.	Isolation of a growth inhibitor from adult connective tissue. Excerpta Med., Vol.15, No.7, Sec.I, p.662, 1961 (abstract).
29.	Parshley, M. S.	Effect of growth inhibitors from adult connective tissue on growth in vitro of a series of human tumors. Excerpta Med., Vol.16, No.9, p.814, 1962.
30.	Parshley, M. S., and I. Mandl	Separation and study of a connective tissue constituent inhibitory to the growth in vitro of tumor cell strains. Proc. Am. Soc. Cancer Res., 4:1:50, 1963.
31.	Parshley, M. S.	Effect of inhibitors from adult connective tissue on growth of a series of human tumors in vitro. Cancer Res. 25:387-399, 1965

1003547068

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

PUBLICATIONS - continued

Mary Stearns Parshley

32.	Parshley, M. S.	Enzymatic destruction of growth inhibitors. Excerpta Med., 19, Sec.I, XXXV, Oct. 1965.
33.	Parshley, M. S., Blackwood, C. E., and Mandl, I.	Specific tumor inhibitors from normal tissue. Proc. Am. Assn. Cancer Res., 6:50, 1965.
34.	Parshley, M. S., and Mandl, I.	Inhibition of malignant cells in vitro by a component of normal adult connective tissue. Nature, 208:800, Nov. 1965.
35.	Parshley, M. S., Mandl, I., and Manahan, J.	Separation and in vitro study of tumor cell inhibitors. Proc. Tissue Culture Assn., San Francisco, June, 1966. In Vitro, 2:124, 1966.
36.	Levi, M., Mandl, I., Parshley, M. S., and Swartz, D. P.	Antibody to papillary cystadenocarcinoma of the ovary. Proc. Pan American Cancer Cytology Congress, May 1967, New York City.
37.	Levi, M., Parshley, M., and Mandl, I.	Antigenicity of ovarian carcinoma cells grown in tissue culture. Proc. Tissue Culture Assn., June 1967, Philadelphia.
38.	Martorelli, Braz, Jr., Parshley, M. S., and Moore, J. G.	Effect of chemotherapeutic agents on a line of human breast carcinoma cells in tissue culture. Proc. Tissue Culture Assn., May 1967, Philadelphia.

10035472069

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

#633 — BOYARSKY

1003547070

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A. September 11, 1967

MEMORANDUM

TO: The committee comprising Dr. Cattell, Chm., Dr. Bing and Dr. Jacobson.

FROM: Robert C. Hockett

SUBJECT: Equipment grant application from Saul Boyarsky, M.D. of Duke University Medical School - NO. 633.

We enclose herewith an application for a grant to purchase an item of major equipment from Dr. Saul Boyarsky of Duke University Medical School in Durham, North Carolina.

In July of 1966, Dr. Boyarsky applied for a grant of $16,907.00 to study the effect of nicotine and other neuropharmacologic agents on ureteral motility. This application was denied by the Board in September, 1966. One consideration may have been the fact that he received a grant for the same purpose from the American Medical Association's Committee for Research on Tobacco and Health on July first of the same year. (My meeting notes do not show for certain whether this fact influenced the S.A.B. decision).

The present application, requesting a great deal more money for a "Hydradjust Table and nine inch image intensifier Vidicon with 500 MA generator cinefluorographic unit" also includes progress reports on the nicotine work which "have demonstrated conclusively that nicotine accelerates the peristaltic rate of the normal and abnormal ureter and increases the force of contraction".

Boyarsky furnishes lengthy curricula vitae of the investigators as well as bibliographies of their publications. Since these were distributed to the same committee with Boyarsky's earlier application, they are being kept on file in the office for reference by the committee chairman.

R. C. H.

1003542071

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

NO. 633
Cf. #562
Denied on 9/17/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application For Research Grant

Date: 31 August, 1967

1. Name of Investigator(s): (include Title and Degrees) Saul Boyarsky, M.D., Prof. of Urology, Director, Urological Research; James F. Glenn, M. D., Prof. of Urology, Chairman Division of Urology; Peregrina Labay, M.D., Research Associate in Urology

2. Institution & Address:

Division of Urology, Department of Surgery
Duke University Medical Center
Durham, North Carolina 27706

3. Short Title of Project:

Nicotine Effect on Ureter in Man: A Possible Therapy for Hydronephrosis.

4. Proposed Starting Date: December 1, 1967

5. Anticipated Duration of this Specific Study: Three years

6. Brief Descripton of Objectives or Specific Aims:

In recent animal experiments we have demonstrated conclusively that nicotine accelerates the peristaltic rate of the normal and abnormal ureter and increases the force of contraction. The renal pelvis and ureter are thereby emptied supernormally by nicotine action.

This nicotine effect has been demonstrated in our laboratory by several in vivo techniques, cinefluorography, peristaltic pressures, surgical observation, as well as in vitro and in abnormal ureter such as diverted, ligated and pharmacologically-blocked ureters. Nicotine produces these effects whether administered by the intravenous, intraarterial, pulmonary or topical routes.

The drug releases catecholamines from sympathetic nerve endings and the adrenal gland in addition to its cholinergic action and its release of antidiuretic hormone. Hence, cigarette smoking may be a "natural" for the treatment of hydronephrosis which is characterized by depressed motility of the renal pelvis and ureter, and excessive collection of urine in the renal pelvis.

The human ureter resembles the dog ureter pharmacologically in every respect we have had an opportunity to study. Our assays of surgical specimens have shown that the dog and human ureters have a similar catecholamine content. The human ureter responds in vitro to the same spectrum of drugs as the dog ureter.

7. Give a Brief Statement of your Working Hypothesis: Since nicotine accelerates ureteral peristalsis in dogs, and the human and dog ureter are similar pharmacologically, the effect of nicotine on the normal and hydronephrotic human ureter would be expected to stimulate peristalsis and to restore tone.

1003542022

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

It is proposed to study normal volunteers and urologic patients by cinefluorography to determine the effect of nicotine and tobacco smoke upon ureteral peristalsis in health and disease.

1) Observation of the ureter during a slow intravenous infusion of concentrated contrast medium will allow us to describe ureteral peristalsis and pelvic peristalsis in terms of rate, rhythm and character of bolus progression.

2) The effect of nicotine administration and tobacco smoking upon this control rate will be determined.

3) Patients with ureteral obstruction, hydronephrosis, dilated renal pelves and surgical lesions of the pelvis will then be studied to note the effect of nicotine upon ureteral and pelvic function in these patients.

4) Ancillary studies will include a) in vitro motility studies; b) fluorometric catecholamine assays of surgically excised ureters and renal pelves; and c) manometric pressure studies of peristalsis will be performed whenever catheters are passed also.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

Duke University Medical Center with its library, faculty and plenitude of research laboratories in related fields provides the background for this study in a university medical center.

See addendum Page 2a.

10. Additional Requirements:

This proposal is actually for a part of our total needs. The requested unit will be an integral part of several larger projects mentioned above and already in progress.

Salaries, supplies and staff are available through these projects. The annual budget is actually between 2 to 3 times the amount of the present request. Our present need is for this equipment, not salaries or supplies. The salaries needed to use the equipment are being funded from other sources, so that the budgetary deletions would represent a "savings" in one sense to the Council for Tobacco Research.

11. Biographical sketches of all principal and professional personnel (append)

12. List of publications: (Five most recent as pertinent) (append)

1003542023

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Addendum to No. 9, Physical Facilities Available

Three hospital services, the Duke Medical Center, Durham Veterans Administration Hospital and the affiliated Watts community Hospital provides some 80-100 urologic beds.

The research laboratories include 2200 sq. ft. in the new Clinical Research II Building and some 1200 sq. ft. in the Bell Building animal laboratories and the Veterans Administration Hospital adjacent to the Duke campus.

At present these laboratories are complete for animal cinefluorography (obsolete for patient use), animal surgery, electromanometry, chemical, histologic and physiologic studies. However, we have not yet funded the cinefluorographic unit for which adequate space has been provided in our new laboratories. These laboratories will pass from the contractor to Duke University within the next 6-8 weeks.

The research staff is one of the largest in any urology department in the country. We have a full-time research professor who is the principal investigator of this project, a full-time research associate, a pharmacologist and a bioengineer in the Division of Urology, a half-time radiologist as well as a collaborating biochemist, (Dr. Norman Kirshner of catecholamine fame) and a physiatrist, Dr. Robert Gregg.

A USPHS academic urology training program supports three pre-residency research fellows and two post-residency research fellows at present engaged in research (three full-time and two half-time). We have five research technicians, two research secretaries, and two research nurses in the urology research laboratory.

As will be detailed below under the additional research support section and in the presentations section, active research programs are in progress in surgical physiology of the ureter, in the area of catecholamine biochemistry, in ureteral neurology, in human diagnostic cinefluorography of the ureter, ureteral reconstruction and pyelonephritis.

Funds are needed for the cinefluorographic unit so that patients can be studied in the urologic facility properly, rather than when an opening on a unit located in the Radiology Department becomes available.

Backing from Dr. Richard Lester, Professor and Chairman of Radiology, is enthusiastic as is evidenced in part by his assignment of Dr. William Barry, Associate Professor of Radiology to Uroradiology.

1003542024

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Addendum to No. 12, List of Publications

Boyarsky, S., Labay, P., Kirshner, N., and Gerber, C.: Does the Ureter Have Nervous Control?, J of Urol., 97:627, 1967 (April).

The Neurogenic Bladder, Saul Boyarsky, M.D. (Ed.) The Williams and Wilkins Co., Baltimore, Maryland, March, 1967.

Boyarsky, S., Labay, P., and Kirshner, N.: Acceleration of Ureteral Peristalsis by Adrenal Compression, Science, 154:669, 1966.

Boyarsky, S., Kirshner, N. and Catacutan-Labay, P.: Catecholamine Content of the Normal Dog Ureter, Invest. Urol., 4:97, 1966.

Boyarsky, S.: Surgical Physiology of the Renal Pelvis and Ureter, Monographs of the Surgical Sciences, Vol. I, pp. 173-213, June, 1964. No. 2

1003542025

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Saul Boyarsky, M.D.
Duke Univ Med Ctr

3.

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Technical		
Sub-Total		
B. Consumable Supplies (list by categories)		
Sub-Total		
C. Other Expenses (itemize)		
Sub-Total		
D. Permanent Equipment (itemize)	$61,950	$61,950
Hydradjust Table		
9" image intensifier Vidicon with 500 MA generator cinefluorographic unit		
E. Overhead (15% of A+B+C)		
Total		$61,950

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature X Saul Boyarsky
Director of Project
(919) 684-2681
Telephone

Signature
Business Officer of the Institution
S. C. Harward
Controller
Telephone

10035420Z9

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Pathophysiology of the Scarred Ureter in Dogs	NIH	$17,500	7/1/67 - 6/30/68
Pathophysiology of Ureteral Obstruction in Man	NIH	17,000	7/1/67- 12/31/67
The Ureter in Pyelonephritis	NIH	14,565	7/1/67- 6/30/68
Ureteral Neurology	NIH	20,410	8/1/67- 7/31/68
Workshop on the Optimal Care of the Neurogenic Bladder Patient	Vocational Rehabilitation Administration	14,966	8/1/67- 7/31/68

Pending

In Vitro Studies of the Ureter	NIH	36,828	7/1/67- 6/31/70
Ureteral Pharmacology	NIH	220,614	7/1/67- 6/30/73
Toxicology and Pharmacology of Ureteropelvic Motility	Army	19,248	7/1/67- 6/30/68

1003547077

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Saul Boyarsky, M.D.
Duke University Med Ctr

Progress Report: Include in sufficient detail, for purposes of evaluation, accomplishments related to the original goals of this project, and information concerning any new lines of investigation which have been followed. (Tables and figures may be included.) Attach additional pages as required.

Effect of Nicotine:

The effect of nicotine upon the function of the renal pelvis and ureter has been established. Ureteral peristaltic frequency is accelerated by a factor of 2 to 5 under optimal doses. This has been demonstrated by the methods of peristaltic pressures, cinefluorography, and direct observation at laparotomy in the dog. Our studies show that nicotine reaches ureteral receptors whether administered topically, intravenously, intraaortically, or by inhalation. The classical biphasic effect of nicotine is observed on the ureter as well as elsewhere, that of preliminary excitation followed by a longer lasting depression of activity. Dose-response curves have not been established completely and definitely yet; work is underway to gather this data.

Localization to Ureter:

Critical experiments have demonstrated that the nicotine effect is entirely separate from that of any antidiuretic effect which may be also introduced. In vitro studies have demonstrated inconstant, unpredictable and weak effect of the drug acting directly on the smooth muscle of the ureter. These studies are continuing.

Nicotine Analogues and Metabolites:

With the cooperation of Dr. Charles H. Jarboe, Assistant Dean and Associate Professor of Pharmacology, University of Louisville, School of Medicine, we have instituted pilot studies on the effects of nicotine monomethiodide and other analogues. There does seem to be ureteral activity inherent in these compounds.

Studies with a batch of cotinine, kindly supplied by Dr. Herbert McKennis, Professor of Pharmacology, the Medical College of Virginia, has shown that these breakdown products of nicotine are inactive.

Mechanism of Action:

The mechanism of action of nicotine on the ureter is under investigation. Studies on the adrenalectomized dog and reserpinized dog, are continuing in order to demonstrate the role of intrinsic catecholamines, vis-a-vis the role of systemic catecholamines secreted by the adrenal gland.

1003547078

Correlation with Renal Effects:

The long series of experiments on the differential effect of topical and nicotinic nerve stimulation has shown that there are overlapping effects between ureteral peristalsis and renal secretion of urine.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Continuing Problems:

At present problems remaining to be investigated include:

The effect of adrenal cortical hormones in supporting muscular contraction

The role of the cholinergic system in ureteral peristalsis.

Continuing collection of data to fill out dose-response curves

Significance of Research:

Elucidation of the renal pelvis and ureter as a delicate neuromuscular mechanism rather than an autonomous muscular tube represents a fundamental advance in Urology and in Ureteral Physiology which should have far reaching diagnostic and therapeutic effects.

Therapy:

Therapeutic uses are suggested by present research and should be welcome. One of the objects of our research is to develop drugs which can be used in the treatment of ureteral colic and similar disorders.

Other Benefits of Research:

The result of this project on our laboratory has been most beneficial. Our group has grown to embrace bioengineer, pharmacologist, and radiologist in addition to the Urology research fellows. The additional equipment has increased the efficiency of our efforts, particularly the tape recorder which has allowed immediate analysis of cinefluorographic experiments and longer periods of observation at lesser expense than was previously possible with cinefluorographic film alone.

1003547079

Presentations:

Does the Ureter Have Nervous Control?, Physiologic, Pharmacologic and Biochemical Evidence for Sympathetic Innervation, Peregrina Labay, American Physiological Society, Houston, Texas, August29-Sept 2, 1966.

Recent Advances in Ureteral Physiology, Saul Boyarsky, Pan Pacific Surgical Association Annual Meeting, Honolulu, Hawaii, Sept 20-28, 1966.

Symposium on Urologic Education, Saul Boyarsky, Landmark Inn, Duke University Sponsor, Div. of Urology, Durham, N.C. August 29-Sept. 2, 1966.

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Presentations: cont'd

Are Ureteral Nerves Functional?, N. Kirshner, S. Boyarsky, P. Labay, C. Gerber, and J. F. Glenn, American Heart Association, New York, New York October 21-23, 1966.

Further Ureteral Neurophysiology Explorations -- Evidence for Nerve Influence on Ureteral Peristalsis, Saul Boyarsky, Spinal Cord Injury Conference, West Roxbury, Massachusetts, Nov. 7-9, 1966.

Ureteral Peristalsis from Conduit to Control Mechanism (Taylor Lecture) Saul Boyarsky, University of Oklahoma, Oklahoma City, Okla., Nov. 17-19, 1966.

Is There a Physiologic Basis for Ureteral Pharmacology?, Saul Boyarsky, Physiology-Pharmacology Seminar, University of Oklahoma, Oklahoma City, Okla., Nov. 17-19, 1966.

Ureteral Pharmacology and Toxicology, Saul Boyarsky, Edgewood Army Arsenal, Edgewood Arsenal, Maryland (March 24, 1967).

EXHIBIT: Physiologic Diagnosis Through Urodynamics, R. D. Ensor, S. Boyarsky, and J. F. Glenn, Southeastern Section of American Urological Association, Hollywood Beach, Florida, April 9-13, 1967.

Ureteral Peristaltic Acceleration by Renal Nerve Stimulation, Peregrina Labay and Saul Boyarsky, American Physiological Society, FASEB, Chicago, Illinois, April 18-19, 1967.

More Evidence for Ureteral Nerve Function and Its Therapeutic Implications, Saul Boyarsky, Peregrina Labay and J.F. Glenn, Annual Meeting of the Society of Genito-Urinary Surgeons, Rye, New York, May, 1967.

EXHIBIT- Physiologic Diagnosis Through Urodynamics, R. Dale Ensor, Saul Boyarsky, and James F. Glenn, American Urological Association, New York May 1967, 1st Prize.

In Vitro Studies of Ureteral Smooth Muscle, J. Malin, S. Boyarsky, and P. Labay, American Urological Association, New York, May 1967.

The Effect of Topical Nicotine on Ureteral Peristalsis, Peregrina Labay, and Saul Boyarsky, American Medical Association 116th Annual Convention, Atlantic City, New Jersey, June 18-22, 1967.

1003547080

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

Presentations cont'd

MOVIE - The Effect of Nicotine on Ureteral Peristalsis - The Experimental Animal.
C. June Pfautz, Peregrina Labay and Saul Boyarsky

presented at the following meetings:

Symposium on Urologic Education, Landmark Inn, Duke Univ. Division of Urology, Sponsor, Durham, N.C., Sept. 1966.

American Urological Association, Annual Meeting, New York, May, 1967.

116th Annual Convention of the American Medical Association, Atlantic City, New Jersey, June 18-22, 1967

Publications:

Does the Ureter Have Nervous Control?, Saul Boyarsky, Peregrina Labay, Norman Kirshner, and Carl Gerber, J. of Urol., 97:627, 1967 (april)

The Effect of Topical Nicotine on Ureteral Peristalsis, Peregrina Labay and Saul Boyarsky, JAMA, 200:209, 1967.

100354708

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000

1003547082

Source: https://www.industrydocuments.ucsf.edu/docs/ptdm0000